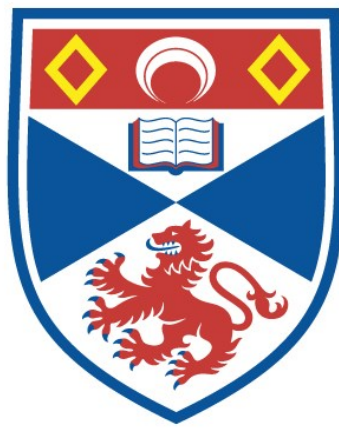


INTERACTIONS BETWEEN THE
PEDUNCULOPONTINE TEGMENTAL NUCLEUS,
MESENCEPHALIC DOPAMINE NEURONES AND THE
STRIATUM

Wendy Louise Inglis

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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**Interactions between the pedunculopontine tegmental nucleus,
mesencephalic dopamine neurones and the striatum**

A thesis submitted to the University of St. Andrews
for the degree of Doctor of Philosophy

by

Wendy Louise Inglis

School of Psychology
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July 1993



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Abstract

The role of the pedunculopontine tegmental nucleus (PPTg) in the control of behaviour is investigated in the experiments in this thesis through (1) the interactions between its ascending cholinergic neurones and dopamine neurones in the substantia nigra and ventral tegmental area; and (2) the involvement of its non-cholinergic neurones in modifying outflow from the striatum.

(1) Stimulation of rat SN by carbachol produces an increase in behaviours for which the animal has a low current baseline rate and a positive predisposition (Winn *et al.*, 1983). This was investigated further by examining first, whether consumption of a palatable food could also be stimulated by intranigral injections of nicotine or acetylcholinesterase inhibitors. Increased feeding was obtained dose-dependently from satiated rats following injection of carbachol, nicotine or neostigmine, but not eserine, into the substantia nigra. Second, it was demonstrated that spontaneous consumption by satiated rats could not be blocked by muscarinic or nicotinic antagonists, although eating stimulated by intranigral neostigmine were attenuated by these antagonists. These data suggest that the cholinergic innervation of substantia nigra is phasic in nature. Third, it was ascertained whether the excitation realised by neostigmine injections into the substantia nigra or ventral tegmental area could support the acquisition of responding for conditioned reinforcement. Injections of neostigmine into the substantia nigra, but not ventral tegmental area, assisted acquisition of the lever-press response and this result is discussed with respect to the significance of habit and expectation on responding.

(2) The PPTg has been viewed as an important striatal output station for several years, primarily due to its proposed function as part of the mesencephalic locomotor region but more recently for its role in incentive behaviours. The validity of these perspectives was tested first by investigating the effects of ibotenate PPTg

lesions on the well-known pattern of events which occur following systemic injections of *d*-amphetamine and apomorphine. The effects of lesions made in the deep mesencephalic nucleus were also investigated as this structure has also been linked with locomotor functions. Neither spontaneous nor drug-induced locomotion was affected by either lesion placement, but PPTg-lesioned rats exhibited abnormal stereotypies. At 3.0 and 5.0 mg·kg⁻¹ *d*-amphetamine these included excessive biting behaviour, predominantly directed at their own forepaws, and they were the only group to score 6 (continuous biting) on the Creese-Iversen scale following apomorphine injections. The role of the PPTg in the mediation of reward-related behaviour was investigated in the conditioned reinforcement paradigm. Ibotenate-lesioned rats responded as frequently as controls on the CR lever, but their pressing was equal on CR and NCR levers. These data are discussed with respect to a role for the non-cholinergic neurones in the PPTg in the mediation of stimulus-reward associations and a possible role for the cholinergic neurones in the integration of such associations back into basal ganglia and cortical circuitry. It is also suggested that the PPTg may have a role in the selection of appropriate and inhibition of inappropriate behaviours.

Abbreviations

2-DG	2-deoxyglucose
6-OHDA	6-hydroxydopamine
ACh	acetyl choline
AChE	acetyl cholinesterase
AD	Alzheimer's disease
AMPH	<i>d</i> -amphetamine sulphate
ANOVA	analysis of variance
APO	apomorphine hydrochloride
BBB	blood-brain barrier
Ch4	cholinergic cells of the nucleus basalis of Meynert
Ch5	cholinergic cells of PPTg and SPTg
Ch6	cholinergic cells of LDTg
ChAT	choline acetyl transferase
CnF	cuneiform nucleus
CPu	caudate putamen
CR	conditioned reinforcer
DA	dopamine
dLGN	dorsolateral geniculate nucleus of thalamus
DOPAC	3,4-dihydroxyphenylacetic acid
DpMe	deep mesencephalic nucleus
DRL	differential reinforcement of low rates
EP	entopeduncular nucleus (internal segment of the GP)
GABA	gamma amino butyric acid
GP	globus pallidus
HRP	horseradish peroxidase
HVA	homovanillic acid
IBO	ibotenic acid

IPN	interpeduncular nucleus
LDTg	laterodorsal tegmental nucleus
LH	lateral hypothalamus
MEA	midbrain extrapyramidal area
MLR	mesencephalic locomotor region
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NA	noradrenaline
NAcc	nucleus accumbens
NADPH	nicotinamide adenine dinucleotide phosphate
NEO	neostigmine methyl sulphate
NMDA	n-methyl-d-aspartate
NO	nitric oxide
PB	phosphate buffer
PBS	phosphate buffered saline
PD	Parkinson's disease
PHA-L	<i>phaseolus</i> leucoagglutinin
PPTg	pedunculopontine tegmental nucleus
PPTg-Ch	cholinergic PPTg
PPTg-nCh	non-cholinergic PPTg
QUIN	quinolinic acid
REM	rapid eye-movement
SC	superior colliculus
scp	superior cerebellar peduncle
SIP	schedule-induced polydipsia
SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra zona reticulata
SP	substance P
SPTg	subpeduncular tegmental nucleus

STn	subthalamic nucleus
TOH	tyrosine hydroxylase
VTA	ventral tegmental area
WGA-HRP	wheatgerm agglutinin conjugated HRP
ZI	zona incerta

1. Brainstem cholinergic nuclei

The cholinergic cells in the pedunculopontine tegmental nucleus (PPTg), subpeduncular tegmental nucleus (SPTg) and laterodorsal tegmental nucleus (LDTg) of the rat brain form a more or less continuous neuronal column in the pons (Figure 1:1). These are the Ch5 (PPTg / SPTg) and Ch6 (LDTg) cell groups as defined by Mesulam and colleagues (Mesulam *et al.*, 1983) and have also been referred to collectively as the pontomesencephalotegmental cholinergic complex (Woolf and Butcher, 1986) and the caudal cholinergic column (Vincent *et al.*, 1986). Part of this cholinergic column corresponds to components of the dorsal tegmental acetylcholinesterase (AChE)-containing pathway described by Shute and Lewis in their pioneering studies on central cholinesterase-containing cells (Shute and Lewis, 1967). As AChE hydrolyses acetylcholine (ACh), Shute and Lewis used AChE histochemistry in combination with radio-frequency lesions and described two separate AChE-positive cell groups, one in the lateral tegmental nucleus and one in an area ventral to the cuneiform nucleus corresponding to the PPTg.

More recent work has identified specific projections from these cholinergic cell populations and it has become clear that these are distinguishable. The recent studies have not used AChE histochemistry as this staining method alone is no longer considered to be definitive for identifying cholinergic cell groups. For example AChE activity is also found in the A6-A7 and A8-A10 noradrenaline- and dopamine-containing cell groups respectively (Lewis and Schon, 1975; Butcher *et al.*, 1975; Lehmann and Fibiger, 1978). Choline acetyltransferase (ChAT), which identifies the essential catalytic enzyme for the formation of ACh, is now regarded as the most reliable marker of cholinergic neurones (Sato *et al.*, 1983). NADPH-diaphorase histochemistry has become an acceptable marker for cholinergic cell groups in the brainstem (Vincent *et al.*, 1983a; Mesulam *et al.*, 1989). NADPH-diaphorase is a nitric oxide (NO) synthase responsible for the calcium/calmodulin-

Figure 1:1

3 sagittal sections taken from the atlas of Paxinos and Watson (1986) illustrating the virtually continuous nature of the LDTg, SPTg and PPTg nuclei. The medio-lateral co-ordinates from the midline are: (A) 0.9 mm; (B) 1.4 mm and (C) 1.9 mm.

dependent formation of citrulline and NO from arginine and in the pons cholinergic neurones appear to be almost alone in producing NO. In a double-labelling study of human brain Mesulam and colleagues (1989) demonstrated that all ChAT-positive neurones in Ch5 and Ch6 stained for diaphorase, although approximately 10% of the diaphorase-positive neurones in this general region were ChAT-negative.

It is clear that non-cholinergic neurones are present amongst the cholinergic cells of the PPTg and LDTg (Sato and Fibiger, 1986; Rye *et al.*, 1987; Lee *et al.*, 1988) and the connections of these will also be discussed. Classical neurotransmitters such as monoamines and γ -aminobutyric acid (GABA) have not been detected in the PPTg or LDTg (Krosaka *et al.*, 1988), although there are GABA terminals there, but there is evidence for glutamate-like immunoreactivity in these regions (Clements and Grant, 1990). A wide range of peptides (for instance substance P, bombesin/gastrin-releasing peptide and corticotrophin-releasing factor) have also been found in both cholinergic and non-cholinergic neurones in these nuclei (Crawley *et al.*, 1985; Vincent *et al.*, 1986).

The pedunculopontine tegmental nucleus

The PPTg was first recognized cytoarchitectonically in normal human material (Jacobsohn, 1909) and was later described in the gorilla (Noback, 1959). Since then there has been a great deal of debate over what defines the PPTg and the controversy has not yet been fully resolved. However, for the purposes of this thesis the PPTg is strictly defined by the stereotaxic atlas of Paxinos and Watson (1986). It consists of a neurochemically and morphologically heterogeneous population of neurones, including the compact sector of the Ch5 cell group (Mesulam *et al.*, 1983; Mesulam *et al.*, 1989), and lies in close association with the ascending limb of the superior cerebellar peduncle (scp) in an area bordered anteriorly by the substantia nigra (SN), posteriorly by the parabrachial nucleus,

dorsally by the deep mesencephalic nucleus and ventrally by the pontine reticular formation (Figure 1:2 [1]).

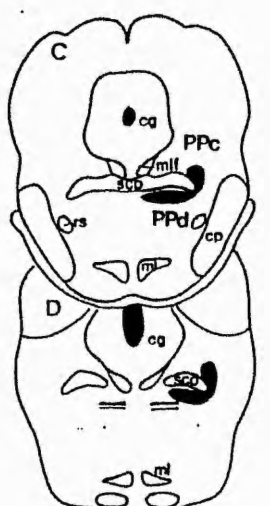
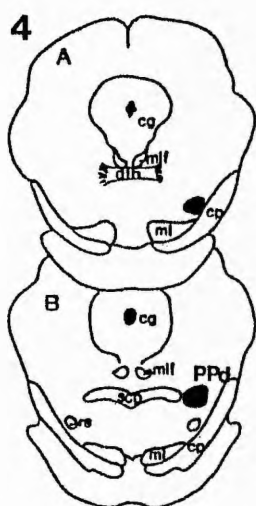
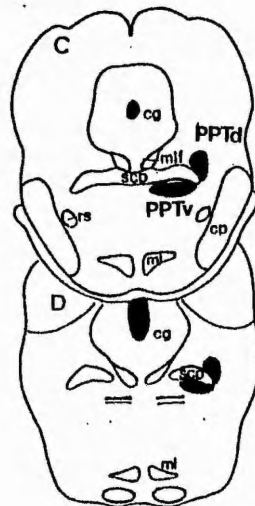
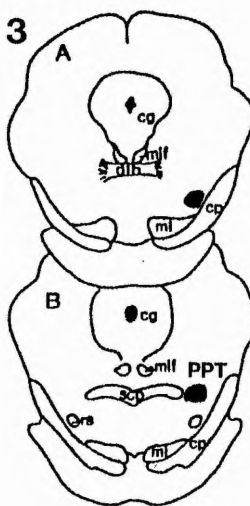
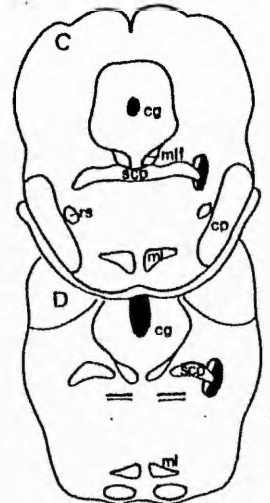
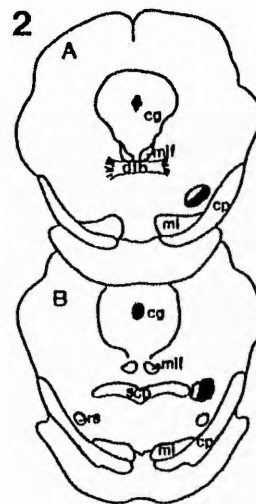
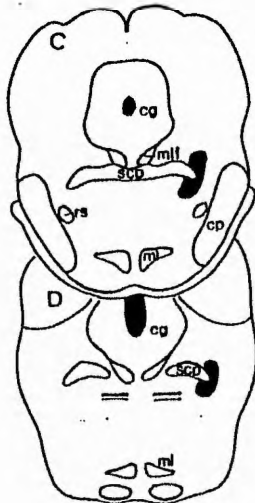
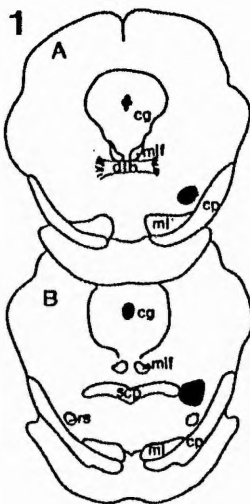
This definition is not identical to those given by other authors. For example, Olszewski and Baxter (1954) distinguished the human PPTg on the basis of cellular density, describing it in terms of two subnuclei (Figure 1:2 [4]). They proposed that the small subnucleus compactus occupied a dorsolateral portion at the caudal half of the nucleus (Olszewski and Baxter, 1954, p.49) and that the remainder of the nucleus constituted the subnucleus dissipatus (Olszewski and Baxter, 1954, pp 49-52). From this definition it is likely that these authors include the subpeduncular tegmental nucleus (SPTg) as part of their pedunclopontine subnucleus dissipatus. While the cholinergic neurones of the SPTg and PPTg make up the Ch5 cell group *en masse* (Mesulam *et al.*, 1989), they can also be considered as separate nuclei (Paxinos and Watson, 1986). Woolf and Butcher (1986) similarly discuss neurones in the SPTg as a ventral extension of the PPTg (Figure 1:2 [3]). Their description of the PPTg indicates that it is "bisected into dorsal and ventral components as a consequence of the lateral encroachment of the superior cerebellar peduncle" (Woolf and Butcher, 1986, p. 606) and at its most posterior point, it is continuous with the LDTg. Their histological pictures (Figures 5-7, pp. 610-612) clearly illustrate that the ventral PPTg cells which they describe are in fact the same as the SPTg as delineated by Paxinos and Watson (1986).

Wainer and colleagues have specifically chosen to deviate from the standard stereotaxic atlas in their definition of the PPTg (Figure 1:2 [2]). This group were the first to describe the PPTg on grounds of its cytoarchitecture in a sub-primate species (Rye *et al.*, 1987). They performed a detailed analysis of the rat mesopontine tegmentum and described a large-celled, cholinergic column extending approximately 1.5 mm from the subcoeruleal region antero-ventrally to the posterior SN. This was cytologically identical with the human PPTg as

Figure 1:2

Coronal illustrations, redrawn from the atlas of Paxinos and Watson (1986), highlighting unilaterally 4 different forms of nomenclature which have been used to define the PPTg. For each, the anterior-posterior co-ordinates from bregma are: (A) -6.8 mm; (B) -7.3 mm; (C) -7.8 mm and (D) -8.3 mm.

1. The PPTg as defined by Paxinos and Watson (1986) and the definition also used for the purposes of experiments in this thesis.
2. The PPTg as defined by Wainer and colleagues (for instance Rye *et al.*, 1987; Lee *et al.*, 1988; Steininger *et al.*, 1992). The black-shaded portion is the PPTg "proper" (ChAT-positive cells) while the adjacent unshaded section is the non-cholinergic PPTg, or midbrain extrapyramidal area.
3. The PPTg as defined by Woolf and Butcher (1986). Posteriorly the compact portion above the scp is called the dorsal part (PPTd) and the portion below the scp is called the ventral part (PPTv), while anteriorly all cells in this nucleus are called the PPT. The PPTv defined here is identical to the SPTg as defined by Paxinos and Watson (1986).
4. The PPTg as defined by Olszewski and Baxter (1954). Posteriorly the densely-populated portion above the scp is called the compact part (PPc) while the less densely-populated portion below the scp and in anterior sections is the pars dissipatus (PPTd). The PPTd includes the cells delineated by Paxinos and Watson (1986) as the SPTg.



described by Jacobsohn (1909). The cholinergic somata were described as multipolar, medium to large in size, ranging from fusiform to triangular in shape, and tending to cluster in groups of 3 to 4. Within the boundaries defined by this cholinergic cell group, Rye and colleagues also described a heterogeneous population of smaller and more lightly stained cells, interdigitated through the larger cholinergic neurones but cytochemically and connectionally distinct from them (Figure 1:2 [2]) . Although they acknowledged at this juncture that the non-cholinergic and cholinergic somata are mixed throughout the nucleus, Wainer has introduced the term "midbrain extrapyramidal area" (MEA) to refer to the non-cholinergic neuronal population. All subsequent research from this group ascribe *only* the cholinergic cells to the PPTg proper, while the non-cholinergic cells are discussed as a separate entity - the MEA (Hallanger *et al.*, 1987; Hallanger and Wainer, 1988; Lee *et al.*, 1988; Steininger *et al.*, 1992). While this practice may be useful for emphasising the differences in connectivity of the separate neurochemically defined neuronal populations, it obscures the likely functional importance of their interdigitation. In an attempt to accommodate both of these aspects, the cholinergic cells of the PPTg will be referred to in this thesis as the PPTg-Ch and the non-cholinergic cells as the PPTg-nCh.

Connections of the PPTg

Early tract tracing experiments did not distinguish between the PPTg-Ch and PPTg-nCh. For example, tracing studies in the rat and cat (Jackson and Crossman, 1981b; Moon-Edley and Graybiel, 1983) used the terminology of Olszewski and Baxter (1954) on a topological basis and defined the PPTg as the region that receives afferents from the basal ganglia and related structures. Since the recent cytoarchitectonic information from Wainer's laboratory (Rye *et al.*, 1987) many of the early anatomical studies in the rat have therefore become less useful: for example it is becoming clear that some of the initially identified afferents and efferents are specific to either PPTg-Ch or PPTg-nCh, while others involve both

types of neurones. The following summary therefore focuses mainly on the recent literature corresponding to connections of the PPTg in the rat, although some early anatomical data from both the rat and other species has also been included where appropriate for comparison.

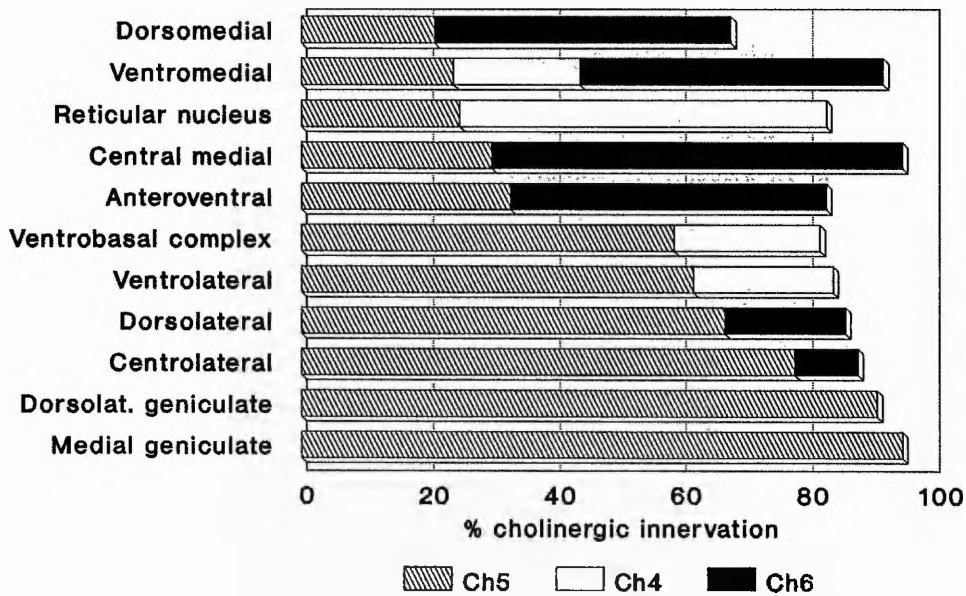
Efferents from the PPTg

Early studies are unanimous in their description of a cholinergic projection from the mesopontine tegmentum to various thalamic nuclei (Shute and Lewis, 1967; Hoover and Jacobowitz, 1979; Mesulam *et al.*, 1983; Sofroniew *et al.*, 1985). Sofroniew estimated that more than 60% of PPTg neurones were retrogradely labelled following a single large thalamic horseradish peroxidase (HRP) injection (Sofroniew *et al.*, 1985) and Wainer and colleagues agree that the majority of cholinergic PPTg neurones project there (Hallanger *et al.*, 1987; Rye *et al.*, 1987). However until relatively recently, studies demonstrating projections to the thalamus have employed large injections of retrograde tracer which labelled all the principal thalamic nuclei in addition to the intralaminar and reticular nuclei (Mesulam *et al.*, 1983; Sofroniew *et al.*, 1985) limiting specific conclusions regarding the terminations of mesopontine cholinergic neurones. Hallanger and colleagues rectified this by comparing the patterns of cholinergic and non-cholinergic thalamic innervation following small injections of retrograde tracer localised to discrete subnuclei (Hallanger *et al.*, 1987). The Ch5 cholinergic cell group was labelled after every thalamic injection suggesting that these cells provide a widespread innervation of the thalamus (Figure 1:3). The medial geniculate, dorsolateral geniculate and ventromedial thalamic nuclei contained moderate numbers of ChAT-positive terminal fields while in other ventral thalamic nuclei they were more sparse. The highest proportion of retrograde labelling after injections to these regions was in the PPTg-Ch. Also, although more than 70% of the cholinergic innervation of the reticular nucleus arises from the basal forebrain (Figure 1:3), Hallanger and colleagues discovered that the remainder originates

Figure 1:3

The relative innervation of the separate thalamic nuclei (data taken from Hallanger *et al.*, 1987) by Ch4 (nucleus basalis of Meynert), Ch5 (PPTg / SPTg) and Ch6 (LDTg) neurones. Ch1, Ch2 and Ch3 have been left out for simplicity. Ch5 neurones innervate every structure, but their strongest innervation is of the dorsolateral and medial geniculate nuclei (vision/hearing), followed closely by the lateral and ventral nuclei (which have close links with motor cortex and basal ganglia circuitry). By comparison, thalamic innervation by the Ch6 neurones is restricted to association "limbic" structures such as the medial and anterior nuclei.

Cholinergic innervation of the thalamus



entirely from the Ch5 cell group, not Ch6. Cholinergic projections to the anterior nuclei, the lateral habenula / dorsomedial nuclei and to the dorsolateral and posterior nuclei came from both Ch5 and Ch6 cell groups (Figure 1:3). Labelling of these structures, except in the cases of dorsolateral and posterior injections, was predominantly from Ch6 neurones. There was also Ch5 input to both the centrolateral and centromedial areas, or intralaminar nuclei. Relative to other thalamic structures these regions received a smaller proportion of their brainstem inputs from the cholinergic nuclei, even though the total number of retrogradely labelled pontine cholinergic fibres was similar.

A limited number of labelled cells in the PPTg-Ch have been reported following retrograde tracer injections into basal forebrain regions (Woolf and Butcher, 1986; Hallanger and Wainer, 1988) and anterograde tracing experiments support this (Hallanger and Wainer, 1988). Numerous ChAT-positive labelled fibres from the PPTg-Ch projected to the lateral septum, while very few innervated the medial septum or the vertical limb of the diagonal band of Broca. There is a substantial projection from the PPTg to the lateral hypothalamus (LH) (Saper and Loewy, 1982; Woolf and Butcher, 1986) and this has recently been specified more clearly as including several posterior PPTg-Ch neurones in addition to a heavy projection from more laterally-placed PPTg-nCh neurones (Hallanger and Wainer, 1988). The PPTg innervation of the ventral pallidal area and the nucleus basalis of Meynert is considered to be predominantly non-cholinergic (Woolf and Butcher, 1986; Hallanger and Wainer, 1988).

HRP injections into the internal segment of the primate globus pallidus (GP) and the feline or rat entopeduncular nucleus (EP) (which are considered to be homologous structures) results in retrograde labelling of cell bodies in the PPTg (Larsen and McBride, 1979; De Vito *et al.*, 1980; Saper and Loewy, 1982; Jackson and Crossman, 1983). These observations have been strengthened by

similar results from anterograde tracing experiments (Graybiel, 1977; Jackson and Crossman, 1980) and although Woolf and Butcher (1986) have claimed that this bilateral projection is cholinergic, Wainer and colleagues have questioned this (Rye *et al.*, 1987; Lee *et al.*, 1988). They argue on the basis of their definition of PPTg-Ch that projections to the EP and GP originate almost exclusively from the smaller PPTg-nCh cells. It is clear that this group have evidence for a strong non-cholinergic projection from the PPTg to the pallidal complex, while also occasionally labelling PPTg-Ch somata (Lee *et al.*, 1988). In the human, Mesulam and colleagues (Mesulam *et al.*, 1983) have indicated that the globus pallidus receives cholinergic innervation, the majority of which originates from brainstem cholinergic nuclei. While species differences may account for this apparent discrepancy, evidence for some form of PPTg-pallidal projection is undisputed and it simply remains questionable to what extent this connection is specific to the PPTg-Ch in the rat.

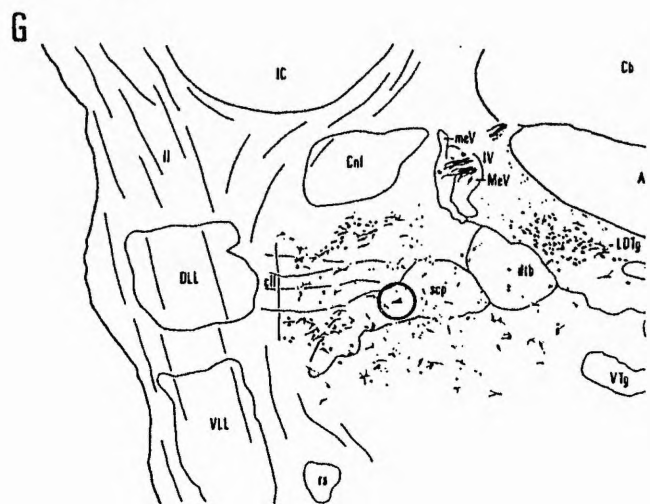
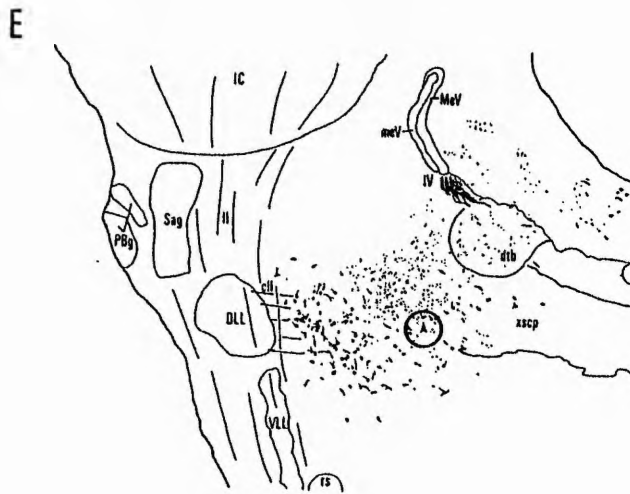
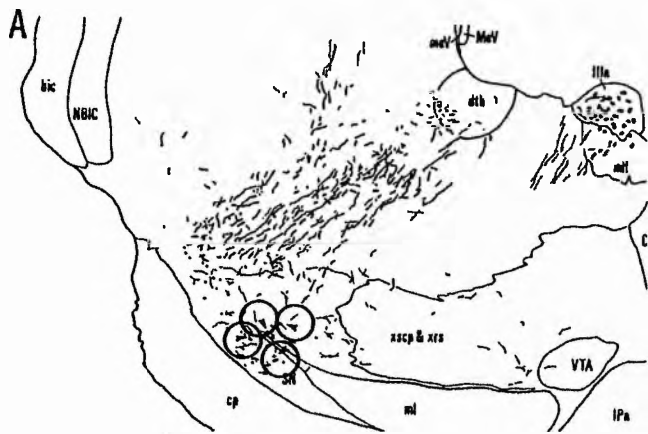
A projection from the PPTg to subthalamic nucleus (STn) was first reported in the cat in an autoradiographic study (Graybiel, 1977) and this was subsequently corroborated using HRP (McBride and Larsen, 1980; Nomura *et al.*, 1980). Evidence for a bilateral pedunculopontine projection to STn was subsequently presented for the primate (Carpenter *et al.*, 1981) and the rat (Saper and Loewy, 1982; Hammond *et al.*, 1983; Jackson and Crossman, 1983; Sugimoto and Hattori, 1984; Rye *et al.*, 1987; Lee *et al.*, 1988). It has been suggested on the basis of electrophysiological evidence that the PPTg-STn fibres are collaterals of PPTg efferents to the pallidal complex (Hammond *et al.*, 1983). Yet again, the nature of the major neurotransmitter in this pathway has been the subject of debate. However it is likely once more that in the rat the majority of neurones originate from the PPTg-nCh, while only a few cells from the PPTg-Ch project to the STn (Lee *et al.*, 1988). The adjacent zona incerta (ZI), a subthalamic structure, also

receives part of its input from the PPTg (Roger and Cadusseau, 1985; Hallanger and Wainer, 1988).

Wainer and colleagues have previously also argued that only the PPTg-nCh projects to the substantia nigra pars compacta (SNc) (Rye *et al.*, 1987; Lee *et al.*, 1988). While these authors have demonstrated a strong non-cholinergic nigral projection from the PPTg, they argue against an extensive body of evidence in favour of a cholinergic PPTg-SNc connection (Saper and Loewy, 1982; Jackson and Crossman, 1983; Moon-Edley and Graybiel, 1983; Sugimoto and Hattori, 1984; Beninato and Spencer, 1987; Gould *et al.*, 1989; Blaha and Winn, 1993). It has been suggested that this conflict can be explained in terms of methodological limitations: Wainer's group used the peroxidase-antiperoxidase staining procedure for their ChAT immunohistochemistry, but apparently did not employ the most sensitive version of that technique available. For instance, Henderson and Greenfield (1987) used the same monoclonal antibody against ChAT as Rye and his colleagues and commented that the "...adoption of a less sensitive procedure for ChAT immunohistochemistry (for example, by omission of the double bridge step in the peroxidase-antiperoxidase method) gave rise to fragmentary or very faint staining for ChAT in axons, dendrites, and small cell bodies..." (p.110, words in parentheses theirs). It is interesting that even with this apparent difficulty, Rye and colleagues did observe a limited number of ChAT-positive neurones in the PPTg which projected to the SNc following infusions of WGA-HRP restricted entirely to nigral tissue (case R21; Figure 28 in Rye *et al.*, 1987 - see Figure 1:4) and that they have since replicated this result (Lee *et al.*, 1988). In the ferret SNc, Bolam and colleagues (Bolam *et al.*, 1991) have demonstrated that ChAT-immunoreactive axons synapse asymmetrically with the dendrites and perikarya of dopaminergic neurones. Although these ChAT-positive fibres could also originate from the Ch6 region, inputs to the SNc from there are as just as controversial. The body of evidence suggests that some form of cholinergic projection to SNc from PPTg

Figure 1:4

Illustrations A, E and G taken from Figure 28 of Rye *et al.* (1987). These camera lucida drawings show ChAT-immunoreactive neurones which were also labelled by WGA-HRP following injected into the substantia nigra (marked by large black arrowheads in the original figure; circles added here for clarity).



does exist, although it may be fairly sparse and many non-cholinergic fibres are also likely to be involved in this projection. Some fibres leave the PPTg by a dorsal lateral pathway and enter the deep and intermediate layers of the superior colliculus (SC) (Hallanger and Wainer, 1988). This pathway has been identified as bilateral (albeit with an ipsilateral predominance) and is likely to be entirely cholinergic (Beninato and Spencer, 1986; Woolf and Butcher, 1986).

It has been suggested that the descending efferents of the mesopontine tegmentum are essential components of multisynaptic pathways to the spinal cord. However, it was only relatively recently that specific cholinergic and non-cholinergic innervation of the medulla and spinal cord was assessed in the rat (Rye *et al.*, 1988). These co-workers demonstrated by both anterograde and retrograde labelling techniques that the PPTg-Ch contributes a large portion of the mesopontine efferents to the medulla. Specifically, they estimated that 18% of the PPTg-Ch neurones were retrogradely labelled from one large WGA-HRP injection into the gigantocellular field of the medulla. A second analysis which demonstrated that more than 50% of the PPTg-Ch cells were labelled from four separate non-overlapping injections in the medulla, suggests that these descending neurones collateralise extensively on their course to the medullary reticular formation. About 10% of the PPTg-Ch neurones project to the rostral ventrolateral medulla, which has been linked to the regulation of blood pressure (Yasui *et al.*, 1990) and another 10% are thought to project to the medioventral medulla which has been identified as a locomotion-inducing area (Skinner *et al.*, 1990b). Only a very small number of neurones from the PPTg-Ch were observed to project as far as the spinal cord (Rye *et al.*, 1988) although some authors have disputed the existence of a PPTg-Ch connection with the spinal cord in either the cat (Moon-Edley and Graybiel, 1983) or rat (Skinner *et al.*, 1990a). However PPTg-nCh neurones have substantial descending projections to the medulla and spinal cord and presumably influence motor or rewarding behaviour through these routes. They provide one of the

heaviest inputs to the gigantocellular portion of the medulla, where the terminal fields from the PPTg-nCh overlap with those from the PPTg-Ch and subcoeruleal region (Rye *et al.*, 1988). Substantial numbers of cells in the rat PPTg-nCh also project directly to the upper thoracic spinal cord (Rye *et al.*, 1988) and these fibres may traverse the ventrolateral funiculus to innervate laminae V-VIII along the entire length of spinal cord (Jones and Yang, 1985).

There also exist a number of less well-documented projections from the PPTg in the rat. These include sparse connections with the caudate-putamen (CPu) (Saper and Loewy, 1982; Woolf and Butcher, 1986; Hallanger and Wainer, 1988) and amygdala (Hallanger and Wainer, 1988). The PPTg also has reciprocal connections with the ventral tegmental area (VTA) (Phillipson, 1978; Jackson and Crossman, 1983; Semba and Fibiger, 1992)

Basal ganglia output and PPTg-nCh

The first experimental study linking the PPTg region with the basal ganglia was by Nauta and Mehler (1966), who demonstrated terminal degeneration in the PPTg following lesions of the medial segment of the ipsilateral GP in the monkey. This has subsequently been confirmed by other anatomical studies and both autoradiographic and electrophysiological experiments (Kim *et al.*, 1976; Harnois and Fillion, 1980; De Vito and Anderson, 1982). Nauta (1979) noted that the pallidotegmental axons in the cat described a relatively cell-sparse field apparently different to the pars compacta of the PPTg. This observation is in good agreement with more recent work of Rye and colleagues (Rye *et al.*, 1987). There is evidence for an EP projection to PPTg from a variety of labelling techniques, in both the cat (Nauta, 1979) and rat (Jackson & Crossman, 1980, 1981b, 1981c). In the rat, this pallidotegmental pathway has been further identified as GABAergic (Moriizumi and Hattori, 1992).

The substantia nigra zona reticulata (SNr) contributes extensive descending projections to widespread regions of the brain stem. Beckstead and colleagues (Beckstead *et al.*, 1979) used autoradiographic tracing in the rat to provide evidence for a nigro-pedunculo-pontine projection, and further support for this derives from retrograde transport of HRP (Jackson and Crossman, 1981b). According to Rye and his colleagues (Rye *et al.*, 1987), the most dense extrapyramidal afferents to the PPTg-nCh in the rat originate from the SNr.

The STn may have a direct ipsilateral projection to the PPTg, although the evidence for this in the literature is contentious: the small size of the STn has made resolution of its connections difficult. In a degeneration study in the monkey, Carpenter and Strominger (1967) demonstrated fibres which terminated in PPTg following STn lesions. However, these fibres were judged to be of pallidal origin since pallido-PPTg fibres run in close proximity to the STn. Subsequently, Nauta and Cole (1978) presented autoradiographic evidence for a specific subthalamo-pedunculo-pontine projection in the monkey, but Carpenter and colleagues (Carpenter *et al.*, 1981) could not verify this clearly using anterograde and retrograde tracing of HRP. Some authors have used anterograde tracers to describe a weak projection in the cat (Moon-Edley and Graybiel, 1983) and rat (Jackson and Crossman, 1981a), while others have contradicted them (Nauta and Cole, 1978; Steininger *et al.*, 1992). In fact several investigators have suggested that anterograde labelling observed in the tegmentum may have resulted from diffusion of tracer into the adjacent ZI or LH (Carpenter *et al.*, 1981; Kita and Kitai, 1987). Specific injections of *phaseolus vulgaris*-Leucoagglutinin (PHA-L) into the ZI or LH have produced a "moderate density" of labelled fibres and terminals in the PPTg (Kita and Kitai, 1987). As most injections of tracer into the STn are likely to label a few neurones in the ZI or LH along the pipette tract, it would be unwise to confirm a subthalamo-pedunculo-pontine projection solely on the basis of anterograde labelling. Specific labelling in the STn has however been

reported following large injections of tracer directly into the rat tegmentum (Jackson and Crossman, 1981a, 1981b). The resulting population of labelled STn neurones was estimated as only 1% of the total population (Hammond *et al.*, 1983). Although this evidence suggests that a small direct projection from the STn to the PPTg-nCh does exist, it is reasonable to assume that the basal ganglia inputs to the PPTg originate mainly from the GP and SNr. Most, if not all, STn neurones send their axons to both the GP and SNr (Van der Kooy and Hattori, 1980) and therefore the STn has undisputed connections with the PPTg, albeit through these intermediate structures.

The laterodorsal tegmental nucleus

The LDTg lies in the central gray of the mesencephalon and pons. It comprises a population of tightly packed cholinergic neurones which have been identified clearly by both ChAT and diaphorase histochemical techniques (Vincent *et al.*, 1983a; Vincent *et al.*, 1986) and classified as the Ch6 cell group (Mesulam *et al.*, 1983). The LDTg lies in the central gray medial to the most posterior part of the PPTg and is bordered posterodorsally by the 4th ventricle and extends anteriorly and medially from the dorsal raphe (Paxinos and Watson, 1986).

Although they are often referred to as a continuous column of neurones in the mesopontine tegmentum, the cholinergic cells of the PPTg and LDTg are distinct from one another on the basis of both cytoarchitecture and connectivity. Cytoarchitectonically they differ in both size and shape: the LDTg perikarya are generally larger than those in the PPTg in maximum soma content (i.e. >25µm) and have an oval or fusiform appearance with dendrites that emerge abruptly from the soma (Woolf and Butcher, 1986; Beninato and Spencer, 1986; Kubota *et al.*, 1992). Many studies have investigated the connectivity of the LDTg in detail using retrograde and anterograde tracers and have indicated that it is a highly differentiated part of the reticular core with strong limbic connections. The

following sections briefly outline the afferent and efferent connections of the LDTg in order specifically to differentiate between the LDTg cholinergic and associated non-cholinergic cells from those of the PPTg.

Connections of the LDTg

Demarcation of cholinergic versus non-cholinergic connections is not yet as clear in the literature for the LDTg as for the PPTg. While it is known that most of the LDTg connections to the tectum, pretectum, thalamus, lateral septum and medial prefrontal cortex are cholinergic (Satoh and Fibiger, 1986), it is likely that afferents and efferents in this region make connection with both types of cell populations as has been demonstrated in the PPTg.

Efferents from the LDTg

There are two major ascending pathways from the LDTg and one descending projection. The short diffuse ascending projection system initially sends its fibres to the medial raphe and the interpeduncular nucleus (IPN) (Woolf and Butcher, 1985; Cornwall *et al.*, 1990; Satoh and Fibiger, 1986) and from here they sweep round the medial lemniscus to link with dopaminergic cell bodies in the midbrain. Some of them travel round the lateral edge of the medial lemniscus to innervate an area just dorsal to the substantia nigra. Although labelled fibres pass close to the SNc, it is not clear whether they actually terminate at its most medial edge (Gould *et al.*, 1989; Cornwall *et al.*, 1990) or simply pass close to it (Satoh and Fibiger, 1986). The rest of this fibre bundle travels over the medial edge of the medial lemniscus and heavily innervates the ventral part of the VTA (Satoh and Fibiger, 1986; Cornwall *et al.*, 1990). A few fibres also cross to the contralateral VTA. Eventually this fibre tract terminates in the lateral mammillary nucleus (Satoh and Fibiger, 1986).

The dorsal tegmental bundle is a long ascending projection which gives rise to many separate fibre pathways throughout the brain. One of these is the tectal pathway, which projects via the periaqueductal gray to enter the deep layers of the SC (Beninato and Spencer, 1986; Satoh and Fibiger, 1986; Hall *et al.*, 1989; Cornwall *et al.*, 1990). Most of these neurones are located in the anterior part of the LDTg and although the projection is considered to be bilateral, it has an ipsilateral tendency similar to the pedunculopontine-collicular projection (Beninato and Spencer, 1986; Cornwall *et al.*, 1990). Some ChAT-positive fibres from this pathway project dorsally to the interstitial magnocellular nucleus of the posterior commissure (Satoh and Fibiger, 1986; Cornwall *et al.*, 1990) and a few axons cross the midline at this point to innervate the contralateral side lightly (Cornwall *et al.*, 1990). This projection also provides sparse innervation of the medial pretectal nucleus.

The dorsal tegmental bundle projects ventrally through the caudal diencephalon and sends groups of fibres dorsally to innervate the parafascicular nucleus and several thalamic nuclei. The LDTg innervation of the thalamus is not as global as its counterpart from the PPTg but some thalamic nuclei receive cholinergic innervation from the LDTg in addition to that from the PPTg. There are relatively heavy LDTg inputs to the anterior, medial and laterodorsal thalamic nuclei and also to the lateral habenula and the intralaminar nuclei (Satoh and Fibiger, 1986; Hallanger *et al.*, 1987; Cornwall *et al.*, 1990; Bolton *et al.*, 1993). There is thought to be a great deal of collateralisation in these projections (Bolton *et al.*, 1993): more than 25% of the cholinergic LDTg neurones which project to the mediodorsal nucleus of the thalamus and approximately 15% of those neurones projecting to the parafascicular nuclei send collaterals to the midline nuclei. A few scattered fibres have additionally been observed in the ventral nucleus of the lateral geniculate (Satoh and Fibiger, 1986).

Some fibres enter the medial forebrain bundle and ascend to the horizontal limb of the diagonal band. At the level of the preoptic area, medially-projecting fibres enter septal regions. Satoh and Fibiger (1986) specified projections to both the lateral and medial septum, but this has been questioned by Hallanger and Wainer (1988). They described both cholinergic and non-cholinergic projections from the LDTg region to the lateral but not medial septum. This has been confirmed by Cornwall and colleagues (Cornwall *et al.*, 1990), who mentioned fibres which crossed the medial septum *en route* for the lateral septum, possibly accounting for the previous discrepancy.

Anterior to this, fibres project medially to innervate the medial bank of the prefrontal (infralimbic and cingulate) cortex (Crawley *et al.*, 1985; Satoh and Fibiger, 1986; Cornwall *et al.*, 1990). This cortical projection, particularly that part which arises from the caudal portion of the LDTg, is bilateral (Cornwall *et al.*, 1990). A very sparse cholinergic projection from the LDTg to the basolateral nucleus of the amygdala has also been described (Hallanger and Wainer, 1988) although others have not found it (Satoh and Fibiger, 1986). There may also be both cholinergic and non-cholinergic inputs from the LDTg to the ventral pallidum / nucleus basalis of Meynert (Hallanger and Wainer, 1988).

Many terminals from the LDTg appear in the lateral hypothalamic area. It has been estimated that between 3 and 16% of retrogradely labelled tegmental neurones from the LH derive from the cholinergic cells of the LDTg (Hallanger and Wainer, 1988). It was also demonstrated in this study that there are many non-cholinergic neurones in the LDTg which project there. Together the targets of these projections form a continuous column throughout the LH in the region of the medial preoptic area and also innervate the supramammillary, lateral mammillary and posterior hypothalamic nuclei (Cornwall *et al.*, 1990) as well as the ZI region (Satoh and Fibiger, 1986). Whether LDTg efferents contact histamine-containing

LH neurones or cortically-projecting LH neurones is unclear, but hypothalamic projections are one of the most prominent components of LDTg outputs.

Descending efferents from the LDTg are not considered to be a significant source of cholinergic innervation of the medulla and spinal cord (Rye *et al.*, 1988). However, projections from the LDTg do innervate a number of brainstem sites known to have visual, orientation and oculomotor functions (Cornwall *et al.*, 1990) and 10% of the LDTg projections in the cat and rat are known to be to the neurones in the gigantocellular tegmental field of the pontine reticular formation, which has been linked to REM sleep (Mitani *et al.*, 1988; Cornwall *et al.*, 1990). Jones and Yang (1985) also suggest that the LDTg may be one of the origins of spinal-projecting neurones in the central gray. However, while these cells may be included in a non-cholinergic region adjacent to the LDTg they are not cholinergic (Standaert *et al.*, 1986).

Afferents to LDTg

Both the orbital and medial prefrontal cortices send direct projections to the LDTg (Vincent *et al.*, 1983b; Wyss and Sripanidkulchai, 1984; Crawley *et al.*, 1985; Satoh and Fibiger, 1986; Semba and Fibiger, 1992) although the retrograde label from the medial prefrontal cortex has been described as sparse (Cornwall *et al.*, 1990). The transmitter for these pathways is probably an excitatory amino acid (Highfield and Grant, 1989). There also exists a population of non-cholinergic neurones in the basal forebrain (nucleus of the diagonal band, substantia innominata, medial preoptic area) which project to the LDTg. These are topographically segregated from those which project to the cortex and it is possible that they contain the neurotransmitter GABA (Cornwall *et al.*, 1990; Semba and Fibiger, 1992). Numerous inputs to the LDTg are from limbic forebrain and midbrain sites, the heaviest of which come from the LH-ZI region and the midbrain central gray (Semba and Fibiger, 1992).

The lateral habenula is one of the major origins of inputs to the LDTg (Satoh and Fibiger, 1986; Hallanger et al., 1987; Semba and Fibiger, 1992) and the IPN also relays information derived from the medial habenula to the LDTg (Woolf and Butcher, 1985; Cornwall *et al.*, 1990). The habenula appears to occupy a position as an output station for the prefrontal cortex, and both the dorsal and ventral divisions of the striatum. Therefore the LDTg receives information directly from both motor and limbic sites via the habenula and also indirectly from septohippocampal sources via the IPN / medial habenula.

Both the VTA and SN (but not the STn or pallidum) project to the LDTg. These may contain a GABAergic component as they inhibit ACh neurones in this nucleus (Semba and Fibiger, 1992). Interestingly, there is thought to be some form of registration between inputs to LDTg from the VTA and LH. The LDTg also receives information from the oculomotor nuclei in the upper brainstem which are important for eye movement co-ordination (Cornwall *et al.*, 1990; Semba and Fibiger, 1992). There are noradrenergic connections from the LC to the LDTg (Kubota *et al.*, 1992; Semba and Fibiger, 1992) and serotonergic projections from the dorsal raphe (Semba and Fibiger, 1992).

Summary of connectional differences between the PPTg and LDTg

Although there are extensive overlaps in the connections of the PPTg and LDTg (for example, in the substantial projections to LH and SC) there are also some very important differences. These may be linked to differences in function, particularly with regard to limbic and motor systems. For example, although both the Ch5 and Ch6 cell groups send fibres to the thalamus, the Ch5 region innervates every thalamic nucleus while the Ch6 cells predominantly innervate limbic thalamic nuclei (Figure 1:3). There are also important limbic-motor differences in the connections of the PPTg and LDTg with dopaminergic cell bodies. While both structures have reciprocal connections with SN and VTA, the LDTg is more strongly connected

with the VTA than SN (Satoh and Fibiger, 1986; Cornwall *et al.*, 1990) and the PPTg is responsible for a large part of the cholinergic innervation of the SN (Blaha and Winn, 1993). The reciprocal cortical connections of the LDTg also link it strongly with the limbic system, while the PPTg is directly involved with motor outflow through its reciprocal connections with the pallidum and STn and its descending projections to the medulla and spinal cord. LDTg connections with lower brainstem regions are predominantly related to visual function.

So while both the PPTg and LDTg are considered to have important activational roles in the reticular system and in relaying output information to pons, medulla and spinal cord, they are likely to be functionally quite different (Figures 1:5, 1:6 and 1:7). Their importance in the control of behavioural processes has not yet been systematically investigated. The experiments in this thesis will examine specific roles of PPTg-Ch and PPTg-nCh neurones in behaviour in an effort to begin to describe the functions of this structure more specifically.

Outline of functions proposed for the PPTg

The target areas of the PPTg-Ch suggest that these neurones have classical reticular functions. Specifically, the ascending projections may be involved (i) in the switching of the thalamus out of burst firing mode and slow wave sleep into single spiking patterns and the waking state (Steriade and Llinas, 1988); (ii) in controlling LH efferents to the cortex which influence the processing of motivationally significant information (Winn *et al.*, 1992); (iii) in stimulating the DA-containing nigrostriatal neurones in the SNc (Blaha and Winn, 1993; Bolam *et al.*, 1991); and (iv) in influencing attentive / orienting processes via projections to the SC and dLGN (Chalupa, 1984; McClurkin *et al.*, 1991). Some of the descending projections from the PPTg-Ch may control muscle tone (Skinner *et al.*, 1990b), while others may have cardiovascular functions (Yasui *et al.*, 1990). In

Figure 1:5

Diagram summarising the major connections of the LDTg. This nucleus has reciprocal connections (filled double arrows) with many limbic structures as well as brainstem oculomotor sites. It also innervates the superior colliculus, limbic nuclei of the thalamus and septo-hippocampal sites without direct reciprocation and receives information from the SNr (unfilled single arrows). Together these suggest a role for the LDTg in processing the motivational significance of visual stimuli.

"Motivational significance of visual stimuli"

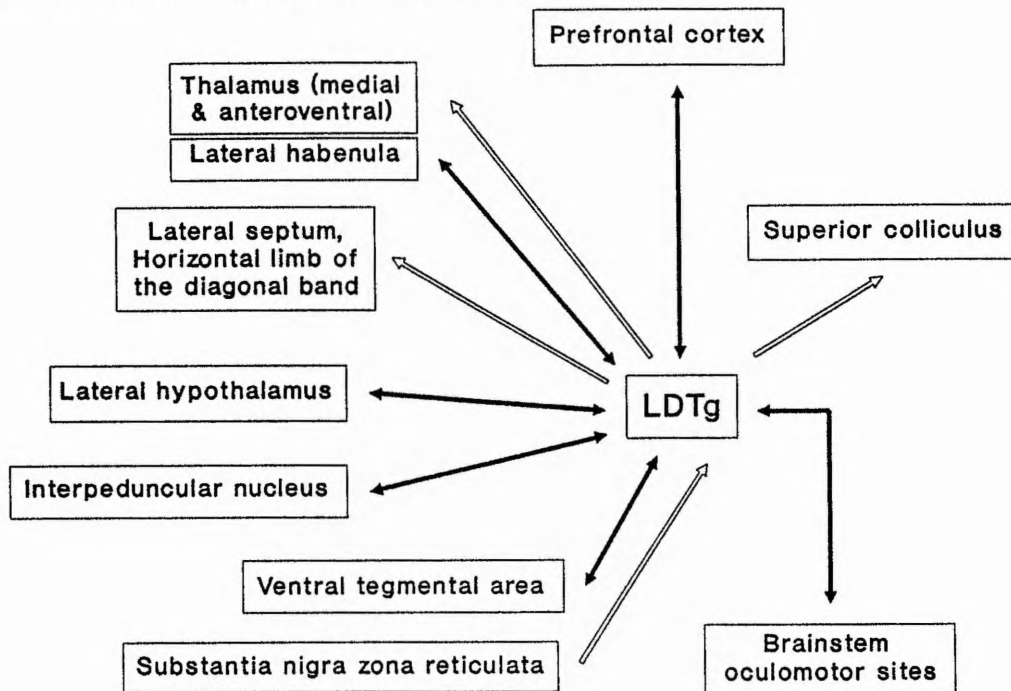


Figure 1:6

Diagram summarising the major targets of PPTg-Ch neurones. These neurones appear to have a role in orienting towards and the processing of significant stimuli and are therefore likely to be directly involved in motor programming.

"Readiness to respond"

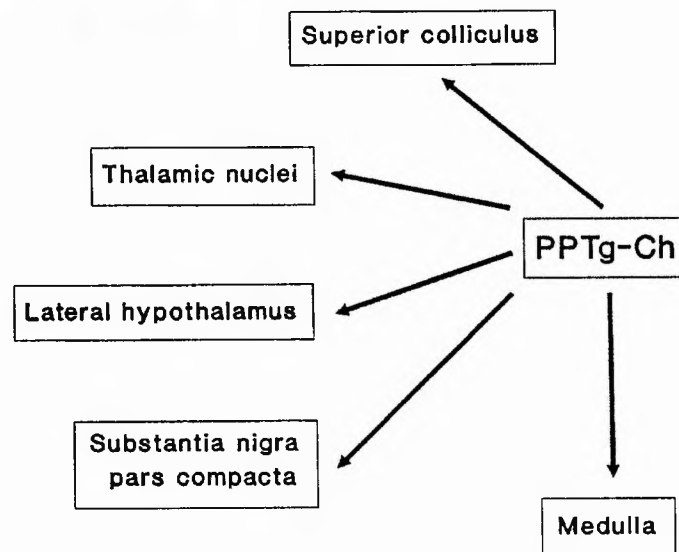
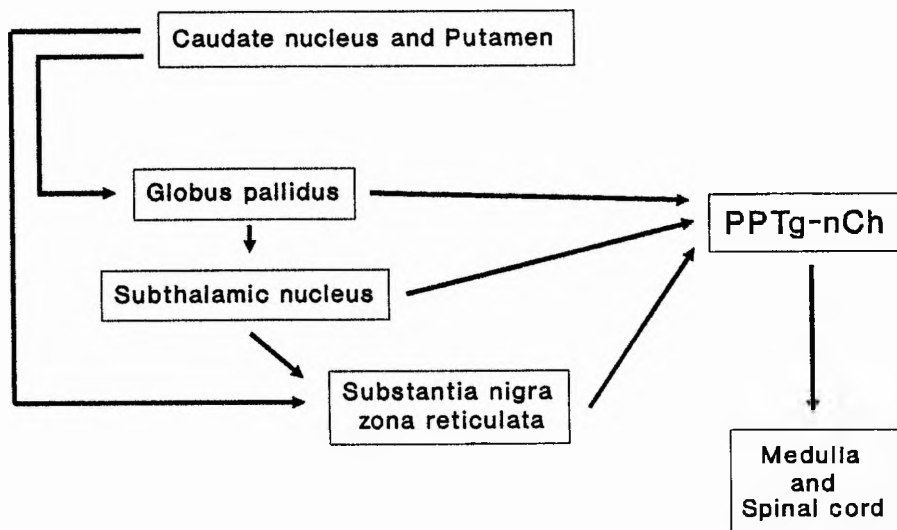


Figure 1:7

Diagram summarising the output of motor information through the PPTg-nCh. Note that the connections with the GP, STn and SN are reciprocal and that output through these nuclei also contains information derived from the nucleus accumbens (not illustrated).

"Motor programming"



more general terms, the PPTg-Ch appears to play an important role in the preparation for action (Figure 1:6).

Non-cholinergic PPTg neurones have been suggested to act as an interface in the mediation of basal ganglia and nigral influences upon lower levels of the neuraxis, particularly in regard to motor systems in the medulla and spinal cord (Mogenson *et al.*, 1980) (Figure 1:7). Much of the information which flows through the PPTg is thought to derive from the striatum and it has been particularly suggested that the PPTg-nCh has a role to play in behaviours mediated by the ventral striatum such as locomotion and reward. The ascending non-cholinergic innervation to basal ganglia and extrapyramidal circuitry may provide a feedback loop for motor outflow information.

The interdigitation of PPTg-Ch and PPTg-nCh neurones is likely to be particularly important for understanding their functions. In addition to the reciprocal nature of PPTg-nCh neuronal connections with extrapyramidal circuitry, integration of output signals with PPTg-Ch neurones can provide forebrain systems with the most up-to-date feedback about specific response selection. Interactions of PPTg-nCh outputs with the PPTg-Ch are also likely to be involved critically in the direct control of action through the extensive connections which PPTg-Ch neurones have with pontine and medullary structures.

The following chapters will elaborate on the functions of the PPTg as they relate to the striatum. The PPTg-Ch has inputs to both the dorsal and ventral striata via cell bodies in the SN and VTA respectively. In return, some striatal output channels funnel their information through the PPTg-nCh *en route* for lower motor regions.

2. Interactions of the PPTg with nigrostriatal dopamine neurones

As outlined in the previous chapter, the PPTg-Ch contacts both the dorsal and ventral striatum via cell bodies located in the SNc and VTA respectively, although it may be most closely linked to A9 (SNc) circuitry while the LDTg may have stronger connections with A10 (VTA) (Sato and Fibiger, 1986; Bolam *et al.*, 1991; Blaha and Winn, 1993). Although the cholinergic contact with the dorsal striatum appears to occur through a simple monosynaptic relay in SNc, it is also influenced by descending information flowing through the SNr. In order to understand these interactions more fully it is essential to consider both the ACh/DAergic and the non-DAergic components of the SN, in addition to the peculiar, ill-understood phenomena of AChE and DA release from nigral dendrites.

The organisation of the substantia nigra

The SN comprises two layers of neuronal somata (Figure 2:1) which share a common domain of dendritic arborizations. The dorsal layer, pars compacta is populated by DA neurones which send axons to the striatum without collateralising (Björklund and Lindvall, 1975). The SNc neurones have very long dendrites (frequently over 500 μm) with few branches, they have a layered organisation and their morphology suggests that they may be able to divide their electrical activity into dendritic and perikaryal domains (Holmes, 1989). They are innervated by cholinergic neurones from the PPTg and LDTg as previously discussed and these contacts may have glutamatergic (Scarnati *et al.*, 1986) and substance P (Vincent *et al.*, 1983b) components.

The ventral portion, zona reticulata is composed of an efferent neuronal population mixed with smaller neurones assumed to be interneurones (Juraska *et al.*, 1987). Afferent projections to this layer arise predominantly from three regions of the basal ganglia (CPu, GP and STn) and from the frontal cortex. The striatonigral

Figure 2:1

2 sections taken from the atlas of Paxinos and Watson (1986) illustrating the separate portions (pars compacta = SNC; zona reticulata = SNR) of the substantia nigra. (A) Sagittal section, positioned +2.4 mm from the midline; (B) Coronal section, positioned -5.3 mm from Bregma.

pathway uses GABA as an inhibitory neurotransmitter (Fisher *et al.*, 1986), substance P and/or substance K as excitatory neurotransmitters (Lee *et al.*, 1986; Bolam and Izzo, 1988) and dynorphin which has variable effects on SN neurones (Vincent *et al.*, 1982; Robertson *et al.*, 1987); these may co-exist in the same striatonigral terminals. The CPu and GP send convergent GABAergic inputs on to the same nigrocollicular (Smith and Bolam, 1991) and nigroreticular neurones (Von Krosigk *et al.*, 1992). In these studies, while pallidal terminals were shown to be large, contain many mitochondria and form asymmetric synapses predominantly with the proximal regions of the neurone, striatal terminals were much smaller and formed symmetric contacts with the distal portions of the same dendritic tree (Figure 2:2). Afferents which project from the frontal cortex to the SNr are likely to be glutamatergic (Kornhuber *et al.*, 1984).

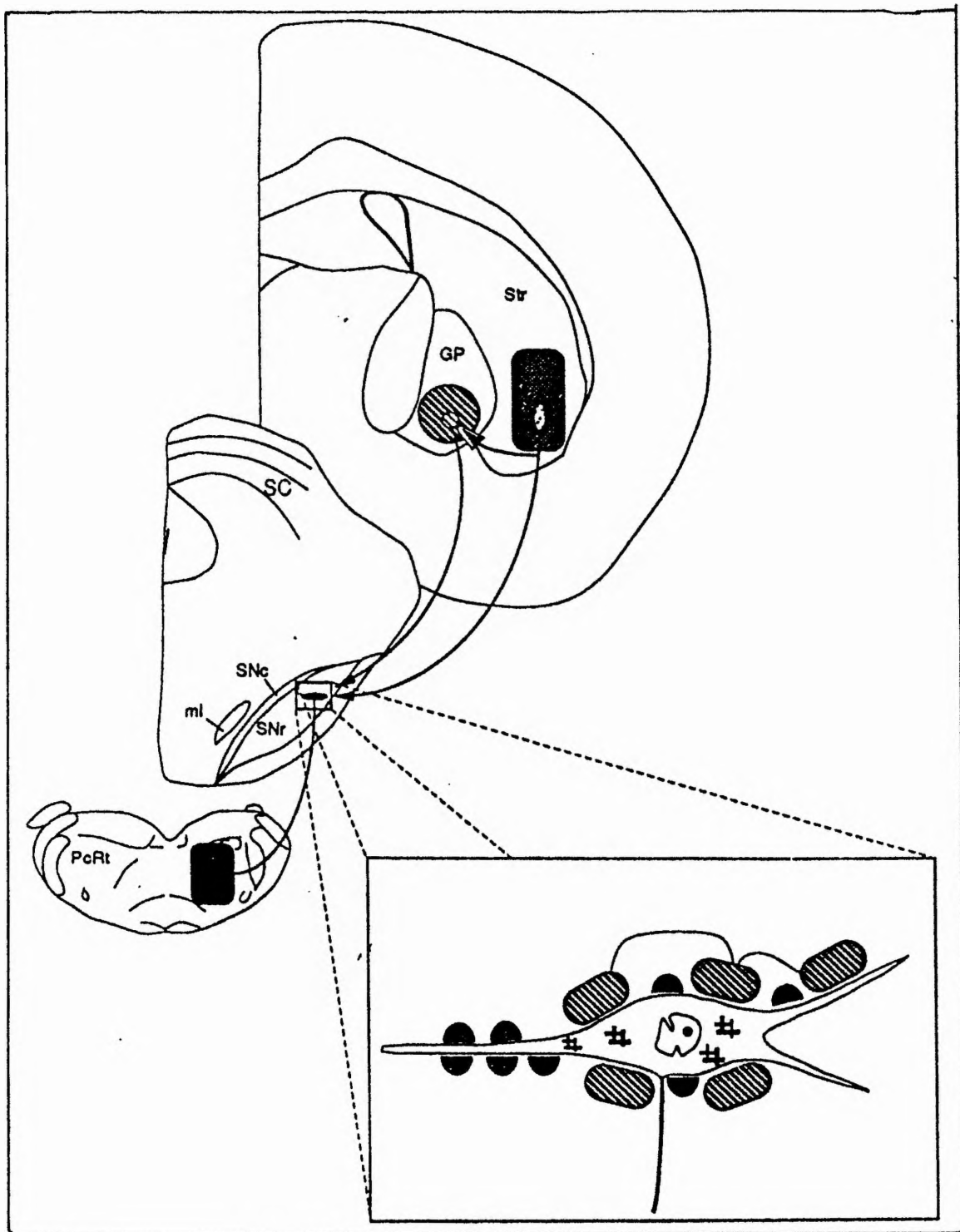
A functional cholinergic system in SNc

The molecules and mechanisms present in the SN indicate that ACh is active as a neurotransmitter there. For example, the amine base choline (Kobayashi *et al.*, 1975), the metabolic enzyme ChAT (Beninato and Spencer, 1987) and the catabolic enzyme AChE (Butcher and Marchand, 1978; Lehmann and Fibiger, 1978) are all found in SN, in addition to a sodium-dependent high affinity choline uptake system and a calcium-dependent release mechanism (Massey and James, 1978). There is extensive other evidence for an excitatory cholinergic role in SN from diverse sources. For example, cholinergic receptors are present on postsynaptic membranes in the SNc where cholinergic neurones make synaptic contact with DA neurones projecting to the dorsal striatum and electrophysiological and behavioural data indicate that cholinergic stimulation of the SNc excites nigrostriatal DA neurones.

ACh/DA synapses. A cholinergic innervation of SN has been clearly demonstrated by histological procedures. Both light and electron microscopy have identified

Figure 2:2

Illustration taken from Von Krosigk *et al.*, 1992 (Figure 7) summarising their anatomical observations regarding convergent innervation of nigral neurones by striatal afferents, both directly and indirectly via the GP. The location of SNr neurones were retrogradely-labelled from the parvicellular reticular formation overlapped with these striatal inputs such that each received significant innervation from both the GP and striatum. This synaptic organisation was also demonstrated for nigrotectal neurones (Smith and Bolam, 1991).



large multipolar ChAT-positive cell bodies and processes which formed asymmetrical synaptic specialisations within nigral boundaries (Beninato and Spencer, 1988; Martínez-Murillo *et al.*, 1989). These ChAT-immunoreactive fibres were most densely distributed in the SNc (Clarke *et al.*, 1987; Henderson and Greenfield, 1987).

Such cholinergic terminals have been shown by direct and indirect methods to make synaptic contact with DAergic neurones in the SN. Indirect histological evidence has been presented from cats with electrolytic lesions of the PPTg. Electron microscopic analysis of SNc 4 days post-lesion indicated degenerating terminals which made asymmetric contact with HRP retrogradely-labelled dendrites of caudate neurones (Tokuno *et al.*, 1988). More recently, direct evidence of synaptic contact with DA neurones has been found in the ferret (Bolam *et al.*, 1991). Bolam and colleagues demonstrated that ChAT-positive fibres entering the SN are closely associated with bundles of TOH-immunoreactive dendrites there. Individual axons at the light microscopic level gave rise to boutons which were in direct contact with DAergic dendrites. Often, as many as ten cholinergic boutons were apposed to a single dendrite and occasionally more than one ChAT-positive axon synapsed on to the same TOH-positive dendrite. At the electron microscopic level, ChAT-positive boutons were often apposed to TOH-immunoreactive dendrites and occasionally also to the perikarya.

Postsynaptic receptors. Significant advances in understanding central ACh circuitry have come from *in vitro* autoradiographic localization studies of ligand-tagged receptors. This technique has clearly demonstrated that [^3H] nicotine and [^3H] ACh, but not [^{125}I] α -bungarotoxin which is thought to label a sub-population of nicotinic receptors, bind densely in the SNc (Clarke *et al.*, 1985b). In addition, M_1 receptor labelling (Cortés and Palacios, 1986; Schwartz, 1986) has been clearly demonstrated there while the presence of M_2 receptors is controversial. M_2 sites in

the SNc have been reported indirectly by the use of the non-specific muscarinic ligand [^3H] quinuclidinyl benzilate ([^3H] QNB) in the presence of pirenzepine to prevent the [^3H] QNB from binding to M_1 receptor sites (Mash and Potter, 1986). However binding by this method supposedly occurs only at the high affinity receptor state (Wang *et al.*, 1989). Mash and Potter attempted to deal with this by including ethylene-diaminetetraacetic acid (EDTA) and N-ethyl maleimide (NEM) in the labelling media to uncouple the high affinity agonist state and therefore presumably allow appropriate visualisation of all M_2 receptors. However in a subsequent study, labelling with a ligand selective for M_2 receptors did not produce specific binding in SNc (Wang *et al.*, 1989). The use of molecular probes has located the m5 molecular muscarinic receptor (thought to correspond to the pharmacological M_1 receptor) to the SN (Weiner *et al.*, 1990). This is further evidence for the presence of M_1 , but not M_2 , receptors at the level of the SNc.

The nicotinic receptors are located in a dense band within the SNc where the perikarya are packed and they are known to be expressed by SNc neurones (Clarke *et al.*, 1985). 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway, which destroyed SNc dopamine neurones, resulted in reduction of [^3H] nicotine labelling (Clarke and Pert, 1985). This technique has also been reported to induce a small loss of muscarinic binding sites (Cross and Waddington, 1980) consistent with the location of muscarinic cholinergic receptors on the cell bodies / dendrites of nigrostriatal DA neurones.

Extracellular single unit recording. It has been demonstrated that ACh and nicotine (applied systemically or locally) can excite neurones in both the SN and VTA (Lichtensteiger *et al.*, 1982; Clarke and Pert, 1985; Grenhoff *et al.*, 1986; Mereu *et al.*, 1987), thought to be DA-containing on the basis of their characteristic slow random firing pattern of 2-8 spikes/sec (Lichtensteiger *et al.*, 1982). Excitation of SNc DA-containing neurones could be blocked by the

centrally-acting nicotinic cholinergic antagonists mecamylamine (Clarke *et al.*, 1985) or dihydro- β -erythroidine (Lichtensteiger *et al.*, 1982), while the muscarinic antagonist atropine caused only a small reduction of the activity induced by ACh (Lichtensteiger *et al.*, 1982). These data suggest that the actions of ACh on nigrostriatal neurones occur principally by nicotinic mechanisms. It has also been demonstrated that kainate infusions into the PPTg at doses which do not induce excitotoxic lesions increase the firing rate of DAergic neurones in the ipsilateral SN (Clarke *et al.*, 1987). This effect is blocked by intravenously administered mecamylamine which again indicates the involvement of nicotinic mechanisms.

Intracellular recording. Recordings *in vitro* from slices of rat mesencephalon have demonstrated that muscarinic mechanisms are also active in SNc (Lacey *et al.*, 1990). These researchers obtained a decrease in spontaneous firing of SNc DA-containing neurones in response to DA application and an increase in response to muscarine. Pirenzepine was shown to reduce the muscarinic action, which is indicative of M_1 -like receptor activation. It is therefore likely that excitation of DAergic nigrostriatal neurones is partly under cholinergic tone mediated by M_1 -like receptors.

Biochemistry. Systemic administration of nicotine has been shown to produce a significant, though modest, enhancement of striatal DA turnover (Lichtensteiger *et al.*, 1982). This was assessed biochemically by measuring the decrease in striatal DA concentration following $0.5\text{mg}\cdot\text{kg}^{-1}$ s.c. nicotine in rats pre-treated with α -methyltyrosine methylester (this inhibits the hydroxylation of tyrosine in catecholamine biosynthesis). DA metabolite concentrations (homovanillic acid [HVA] and 3,4-dihydroxyphenylacetic acid [DOPAC]) were also measured: enzyme assays and high pressure liquid chromatography analyses were carried out using brains frozen on dry ice 20 min following nicotinic injection. Levels of caudate HVA were significantly increased following systemic nicotine treatment.

Chronoamperometry and microdialysis. DA efflux in the dorsal striatum was measured by chronoamperometry following infusions of carbachol, nicotine or neostigmine to the SNc (Blaha and Winn, 1993). A dose-dependent increase in DA measured by an increase in the chronoamperometric signal was observed and the stimulatory effects of neostigmine on DA efflux were confirmed by *in vivo* microdialysis. Quinolinic acid lesions of the PPTg which destroy the cholinergic neurones there relatively selectively (Rugg *et al.*, 1992) attenuated the increased DA efflux in the striatum following intranigral neostigmine as compared with sham-operated and intact control rats. In contrast, striatal DA efflux was enhanced compared with controls following intranigral infusions of nicotine. This result suggests development of post-synaptic receptor supersensitivity following afferent degeneration and is further evidence of a functional connection between the PPTg and CPu via the SNc.

Behavioural studies. Behavioural data have long supported the presence of a cholinergic connection in SN. As early as 1966, Smelik and Ernst described "compulsive" stereotyped gnawing following application of 30µg crystalline physostigmine salicylate (a ChE inhibitor) to SN by pushing it down a guide cannula with a stylet. It was suggested that this might be due to DAergic mechanisms (Smelik and Ernst, 1966). Rats pretreated with i.p. atropine did not show this gnawing behaviour, which suggested that it required a muscarinic mechanism.

Further demonstration of a cholinergic function in SN subsequently came from direct stimulation with carbamylcholine chloride (carbachol). For example, microinjection of 10-40 µg carbachol into the SN of Wistar rats evoked stereotyped behaviour not distinguishable by sight from that evoked by systemic apomorphine (Decsi *et al.*, 1978). This could also be reversed by systemic pretreatment with atropine. In an operant task, rats injected with doses of 1.0 and

5.0 μ g carbachol have been shown to induce a behavioural stereotypy characterised by chewing, gnawing and biting of the levers without any attempt to retrieve the food pellets (Winn and Redgrave, 1979). Microinjection of 2 μ g carbachol into the SN of rabbits increased the alert EEG and evoked epileptic EEG discharges accompanied by increased walking, sniffing, head turning, ipsilateral rotation and abnormal forepaw movements (Wolfarth *et al.*, 1978). Similar effects have also been achieved with intranigral injections of ACh or neostigmine and were blocked by intranigral atropine (Wolfarth *et al.*, 1974).

Microinjection of lower, pharmacologically relevant, doses have allowed observation of more "normal" behaviours. At lower doses, microinjection of cholinergic substances (for instance, 0.1 or 0.5 μ g carbachol; 2.5, 5.0 or 10.0 μ g physostigmine sulphate alone or in conjunction with ACh) into the rat anterior SN produced increased consumption of spaghetti but not lab chow in satiated rats (Winn and Redgrave, 1979; Winn and Redgrave, 1981). Feeding has also been demonstrated in an operant task where 0.5 μ g carbachol increased lever pressing for and consumption of food pellets (Winn and Redgrave, 1979). Feeding in response to an ACh/eserine mixture has been blocked by a preinjection into the SN of atropine (Winn *et al.*, 1983). Drinking of tap water in these studies was unaffected, although intranigral carbachol can stimulate drinking of saccharin solution (Winn, 1990). At these doses of carbachol there is no effect on other behaviours such as gnawing, drinking, locomotion, grooming, sniffing or rearing (Winn, 1990; Winn *et al.*, 1983). Carbachol microinjections have, however, been shown to affect sexual behaviour in male rats (Winn, 1990).

The strength of the behavioural response to cholinergic stimulation of the anterior SN has been shown to be proportional to the proximity of the injection site to the SNc (Winn and Redgrave, 1979) and the behavioural effects of these injections are thought to depend on stimulation of DA-containing neurones in the SNc. This

suggestion is not only complemented by the compelling evidence from other experimental sources previously outlined, but there are also directly supportive behavioural data. Firstly, consumption of spaghetti, but not drinking, locomotion or stereotypy was observed in satiated rats following microinjection of low doses of *d*-amphetamine (AMPH) into the dorsal striatum (Winn *et al.*, 1982). Secondly, systemic injections of non-sedative doses of the DA antagonist haloperidol block behaviour stimulated by injection of carbachol into SNc (Taha and Redgrave, 1980). Thirdly, it has been demonstrated that increased feeding following stimulation of the anterior SN by carbachol is abolished after a unilateral 6-OHDA lesion of the nigrostriatal pathway, made at the level of the LH, which caused a 50% reduction in the DA content of the dorsal but not ventral striatum (Parker *et al.*, 1991).

Cholinergic stimulation of SN can best be described as eliciting dose-dependent increases in behaviours for which the animal has both a low current baseline rate of activity and a positive predisposition (Winn *et al.*, 1983). The behavioural specificity of cholinergic stimulation of SN should be emphasized: intranigral carbachol elicits no unconditioned responses other than feeding on palatable food, drinking of saccharin solution, or changes in sexual behaviour. This is in marked contrast to other elicitors of feeding such as tail pinch or electrical stimulation of the lateral hypothalamus. In each of these the response appears to be stimulus-bound, as the absence of food induces other behaviours such as gnawing (Valenstein *et al.*, 1968; Robbins and Fray, 1980). Behaviours elicited following cholinergic stimulation of SN are also different to those following stimulation by other neurotransmitters present there. For example, intranigral GABA suppresses the intake of sweetened milk (Kelly *et al.*, 1977) while substance P increases locomotion, grooming and sniffing (Kelley and Iversen, 1978).

Together, these behavioural data suggest specific functions for ACh in SN which are likely to be related to DAergic function in the dorsal striatum. Electrophysiological data show that neuronal firing in the SNc immediately precedes movement (De Long *et al.*, 1983) and that activating rather than immobilising stimuli promote single unit activity in nigrostriatal DA neurones (Chiodo *et al.*, 1979). However, activation of these DA neurones by ACh is behaviourally specific and is not moderated by the environment: there is no generalised stimulatory effect on unconditioned behaviour following carbachol or ACh/physostigmine injections into SN. Although cholinergic stimulation of SN clearly does increase levels of DA in the dorsal striatum (Blaha and Winn, 1993), the behavioural effects of this increase contrast markedly from those following direct DA release there by AMPH, which has more generalised psychostimulant actions (Lyon and Robbins, 1975). AMPH is known to induce massive efflux of DA from neuronal terminals while also blocking its re-uptake. Therefore, it is possible that behaviours elicited by cholinergic activation of nigrostriatal DA are more readily tempered by firing patterns in other striatal afferents than the massive extracellular DA elevations generated by AMPH infusions. It is also likely that ongoing cholinergic excitation of nigrostriatal DA can be modulated through feedback from the SNr and this possibility will be discussed later.

Topographical organisation of the SNr

Anatomical studies in the rat have demonstrated that innervation of the SNr from the dorsal striatum, GP and STn is arranged in a clear topographical framework. First, for most of the striatonigral connections mediolateral relationships are maintained, but there is a dorsoventral inversion such that dorsal regions of the striatum project to ventral regions of the SNr and projections of ventral CPU origin project to the dorsal SNr (Gerfen, 1985; Tulloch *et al.*, 1978); second, the majority of the pallidonigral projections arise from the lateral GP and display a rostral to medial and caudal to lateral topography (Smith and Bolam, 1989); and third, in the

projections to the SNr from the STn, both mediolateral and dorsoventral topographies are preserved (Kita and Kitai, 1987). As discussed previously, afferents to the SNr from the striatum and pallidum converge on to the same neurones and the synaptic organisation (pallidal → proximal; striatal → distal) is more or less constant (Smith and Bolam, 1991; Von Krosigk *et al.*, 1992).

The efferents from SNr project to a variety of destinations (for example SC, thalamus and brainstem) and until recently the nigrothalamic projection was considered to be segregated regionally in the SNr from the nigrotectal pathway (Bentivoglio *et al.*, 1979; Faull and Mehler, 1978). However electrophysiological evidence and recent tracing studies have indicated extensive collateralization of nigral efferents. Many of the same SNr cells can be activated by antidromic stimulation in the thalamus and tectum (Chevalier *et al.*, 1981; Chevalier *et al.*, 1985; Deniau and Chevalier, 1985) and tracers studies have demonstrated that both nigrotectal and nigrothalamic neurones are found in widespread regions of the SNr (Herkenham, 1979; Gerfen *et al.*, 1982; Redgrave *et al.*, 1992). In addition, the nigrotectal projection (to the intermediate layers of the SC) is known to have at least two components: neurones in the dorsolateral SNr project to the rostromedial SC (Redgrave *et al.*, 1992) while cells in the ventromedial SNr project to caudomedial SC (Beckstead *et al.*, 1979). Similarly, there are separate output channels for the thalamus: while the dorsal SNr projects to the ventromedial nucleus of the thalamus (Herkenham, 1979), there are at least two output channels from the SNr to the mediodorsal nucleus of the thalamus: the dorsolateral SNr projects to the lateral mediodorsal nucleus and the ventral and medial portions of the SNr project to the medial portion of the mediodorsal nucleus (Gerfen *et al.*, 1982). It is as yet unclear whether the nigral innervation of brainstem regions exhibits similar channelled organization, although Von Krosigk and Smith (1990) have demonstrated clearly that the dorsolateral SNr connects with the lateral pontine and medullary reticular formation.

Neuronal interactions of SNc and SNr

The organisation of the SN is reminiscent of the PPTg in that both of these structures comprise two morphologically, connectionally and neurochemically distinct systems. Indeed the SNc carries ascending information analogous to the PPTg-Ch (although of course there are far more PPTg-Ch efferents, some of which travel to more caudal regions), while much of the information flowing through the SNr, like the PPTg-nCh, is descending "motor outflow" information. Although the neuronal populations in the SN (and indeed the PPTg) convey separate streams of neuronal information, their somata are clearly interconnected such that these streams of information can influence each other. This sort of interaction must manifest itself behaviourally. For instance neurones in the SNc may influence those in the SNr, providing the SNr output channels with the most recent information about changes in the environment and therefore permitting some degree of flexibility in final response selection. The neuronal interaction is also likely to allow SNr modulation of SNc firing to provide recurrent modulation of the overall excitatory influence on striatal neurones in respect of the motor outflow information which is carried in the SNr. In addition to the direct effects of neuronal interactions in the SN, SNc neurones are also known to release AChE and DA from their dendrites (Greenfield *et al.*, 1983; Geffen *et al.*, 1976). This release has been the subject of a great deal of speculation and discussion in terms of their effects on neuronal firing patterns and behaviour.

AChE release in SN. Historically, AChE release in SN was the subject of a great deal of speculation, initially because the volume of AChE present in SN was considered to be far more than should be required solely for its role in the hydrolysis of ACh and latterly because it was believed that there was no cholinergic innervation of SN.

Much of the AChE present in the CSF has been shown to originate from nigrostriatal neurones (Greenfield and Smith, 1979). It is released from somata and dendrites within the SNc in addition to caudate nucleus terminals (Greenfield *et al.*, 1983). Dendritic release of AChE in SN is apparently unrelated to frequency of somatic discharge of nigrostriatal neurones (Greenfield and Weston, 1984). However as AChE release in the SN of the awake animal was more pulsatile than in the anaesthetised state it is likely that its release is sensitive to the bombardment of incoming afferent signals (Taylor *et al.*, 1990). These signals could relate to firing from cholinergic inputs to the SNc and/or to firing of neurones in the SNr. In addition, some release could be due to signals from outwith actual synapses: some Ch6 fibres may pass very close to the SN without actually synapsing there (Sato and Fibiger, 1986). Vizi and colleagues (Vizi *et al.*, 1983) have shown that ACh can be released non-synaptically from axons in the periphery. If this also occurred centrally then passing cholinergic fibres might release ACh to act on SNc receptors. There are no dendro-dendritic synapses in SNc, diffusion of substances would occur in a relatively non-restricted manner. ACh reaching nigral neurones from outwith the synapse might be involved in the release of AChE and then itself become the substrate for hydrolysis by the AChE. Another possible controller of released AChE, not mutually exclusive to that outlined above is from the GABAergic output through the SNr. There is evidence that release of AChE from some nigral neurones is increased by gamma-hydroxybutyrate (GHB) (Harris *et al.*, 1989), which can be formed from GABA. Although the production of GHB reflects only a minor route for the metabolism of GABA and exists in very small concentrations, it may nevertheless have an important role in release of AChE.

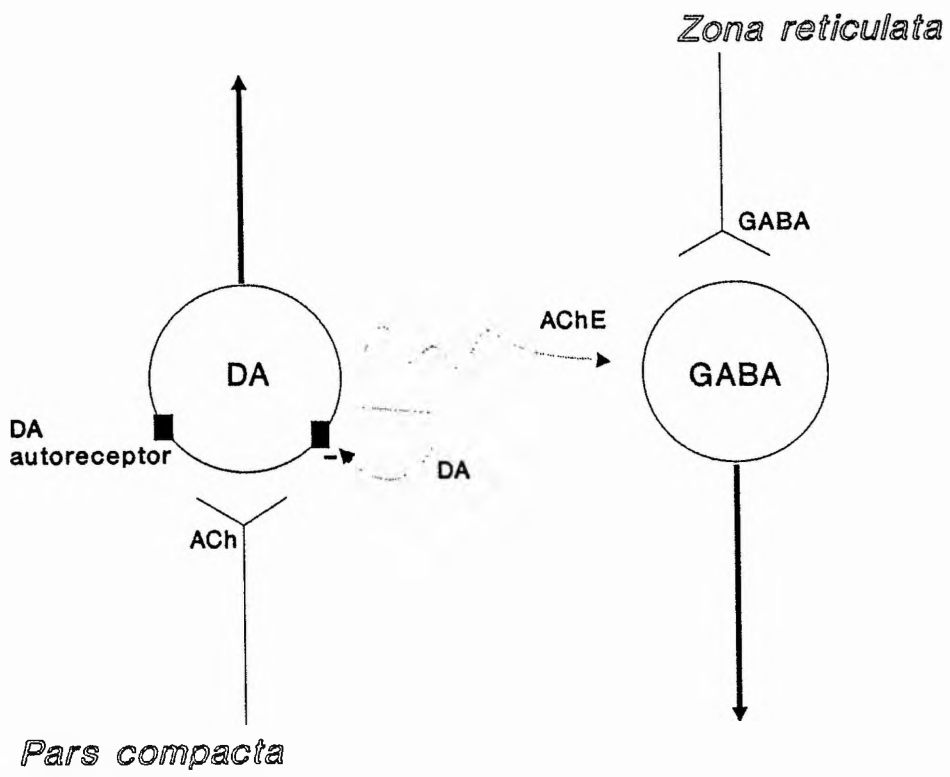
In SN, local microinfusion *in vivo* of AChE but not non-specific cholinesterase depressed both spontaneous and stimulus-linked firing of identified SNc neurones (Greenfield *et al.*, 1981). This is supportive, but not compelling, of a role for AChE in SN in the hydrolysis of ACh. Co-existence of substance P with ACh in the

ascending Ch5 and Ch6 pathways (Vincent *et al.*, 1983b) and with GABA in striatonigral neurones (Lee *et al.*, 1986; Bolam and Izzo, 1988) may however indicate another function for both membrane-bound and released AChE in SN. Chubb and his colleagues have shown that *in vitro* AChE has peptidase activity towards SP (Chubb *et al.*, 1980). In addition, behavioural evidence is supportive of a role for AChE in GABAergic function in SNr (Figure 2:3). Rats treated with i.p. AMPH followed by AChE microinjections to the SN rotated away from the side of the AChE injection (Greenfield *et al.*, 1984). Although microinjected AChE would hydrolyse endogenous ACh in SNc, the decrease in activity in the nigrostriatal pathway which this would induce would be negligible compared with the effects of systemic AMPH. The rotation was also unlikely to be directly related to cholinergic function in SN because rotation away from the side of the AChE injection would be the same as rotation *towards* the side of greatest additive nigrostriatal neuronal activity (AMPH + normal ACh vs. AMPH alone). However rats with unilateral 6-OHDA lesions of the nigrostriatal pathway rotate towards the side of the lesion following AMPH treatment (*away from* the side of greatest neuronal activity). The circling effects of AChE in SN are therefore more likely to be related to GABAergic function in SNr.

DA release in SN. Björklund and Lindvall (1975) demonstrated that DAergic neurones in SN have numerous small swellings and varicosities along their dendrites which contain high amounts of DA. It is now well established that DA is released spontaneously from dendrites of nigrostriatal neurones and that such release is Ca^{2+} -dependent and insensitive to tetrodotoxin (Cheramy *et al.*, 1981). The relationship between dendritic release and neuronal discharge however is unclear: it is not related to the firing of nigrostriatal neurones as it is enhanced by both serotonin and ACh (Glowinski and Cheramy, 1981), which have opposite effects on the firing of SNc neurones (Dray *et al.*, 1976; Walker *et al.*, 1976), and

Figure 2:3

Diagram illustrating the possible sites of action for the acetylcholinesterase (AChE) and dopamine (DA) released from the pars compacta neurones in substantia nigra. Released AChE may modulate outflow of information through the zona reticulata while released DA might directly inhibit the firing of nigrostriatal neurones.



is decreased by substance P which like ACh has excitatory effects on SNc neuronal firing (Walker *et al.*, 1976; Glowinski and Cheramy, 1981).

However, potential roles for released DA in SN may be less difficult to ascertain. First of all, a DA-sensitive adenylate cyclase has been localised pre-synaptically to nigral terminals of neurones originating from the striatum and GP (Iversen, 1977) and DA can stimulate the release of GABA *in vitro* from presumed GABAergic axon terminals within the SN (Reubi *et al.*, 1977). GABA iontophoresed onto identified SNc neurones produces a rapid and marked inhibition of their firing (Scarnati and Pacitti, 1982) as does DA itself (Aghajanian and Bunney, 1973). Firing of SNc neurones in the monkey can also be depressed by APO (Aebischer and Schultz, 1984) and AMPH (Rebec and Groves, 1975). These data suggest a role for released DA in inhibiting the firing of SNc neurones both directly via an autoreceptor and indirectly through release of GABA from SNr neurones (Figure 2:3).

Summary of transmission in SN

In summary, it appears that DAergic SNc neurones which innervate the dorsal striatum are activated by ACh predominantly arising from the PPTg-Ch. Firing of SNc neurones may be exclusively inhibited via GABA released from the SNr, although their firing patterns may also be gated via the actions of DA, released from the SNc, on D₂ autoreceptors. The SNr has topographically organised inputs from dorsal striatum, subthalamic nucleus, globus pallidus and frontal cortex and sends channelled output to the superior colliculus, thalamus and brainstem. Released AChE may affect this GABAergic transmission in the SNr. The data which have been discussed here in relation to neurotransmission in the SN have not addressed a role for SNr interneurones which have been clearly visualised in Golgi stained slices (Juraska *et al.*, 1987). However, since the small cells of the SNr have

not yet been neurochemically defined, it is difficult to hypothesize what their function might be.

3. Role of central dopamine systems

Organisation of inputs and outputs of the striatum

The striatum comprises the caudate nucleus and putamen (CPu) (dorsal striatum) and the nucleus accumbens (NAcc) (part of the ventral striatum). Although these are cytologically and neurochemically similar regions, they have been delineated separate territories of the striatal complex on the basis of their connections. In addition, both the CPu and NAcc have separated divisions, identifiable by input/output streams. For instance on the basis of these the NAcc is comprised of three distinct subterritories: core, shell and rostral pole (Zaborsky *et al.*, 1985).

Afferents and efferents of striatal subcompartments

The dorsal striatum receives DAergic input mainly from SNc (Björklund and Lindvall, 1975) and also receives innervation from the centromedial nucleus of the thalamus. Cortical afferents to the dorsal striatum are topographically organized. For instance, the frontal eye fields and the visual cortex in the temporal lobe project to the body and tail of the caudate respectively, while the head of the caudate receives input which includes the prefrontal cortex (Caan *et al.*, 1984; Alexander *et al.*, 1986). The outputs from the CPu contact brainstem motor systems through their projections to the SNr via the GP and STn (Tulloch *et al.*, 1978; Gerfen, 1985).

The entire ventral striatum receives a projection from the basal and lateral nuclei of the amygdala, the ventral pallidum and from the subiculum (Brog *et al.*, 1991; Zahm and Brog, 1992). These afferents overlap the terminal distribution of DAergic terminals which arise predominantly from the VTA, but also sparsely from the A8 and A9 regions. The core also receives inputs from the STn and GP, while the shell has inputs from the bed nucleus of the stria terminalis, preoptic area, lateral hypothalamus and medial amygdala. Likewise, cortical inputs exhibit

core/shell specificity (Zahm and Brog, 1992). Projections from the prelimbic, anterior cingulate and dorsal agranular insular cortices are mainly to the core, while infralimbic, ventral agranular insular and piriform cortices project mainly to the shell. The afferent projections to the rostral pole comprise a combination of core and shell innervation (Brog *et al.*, 1991).

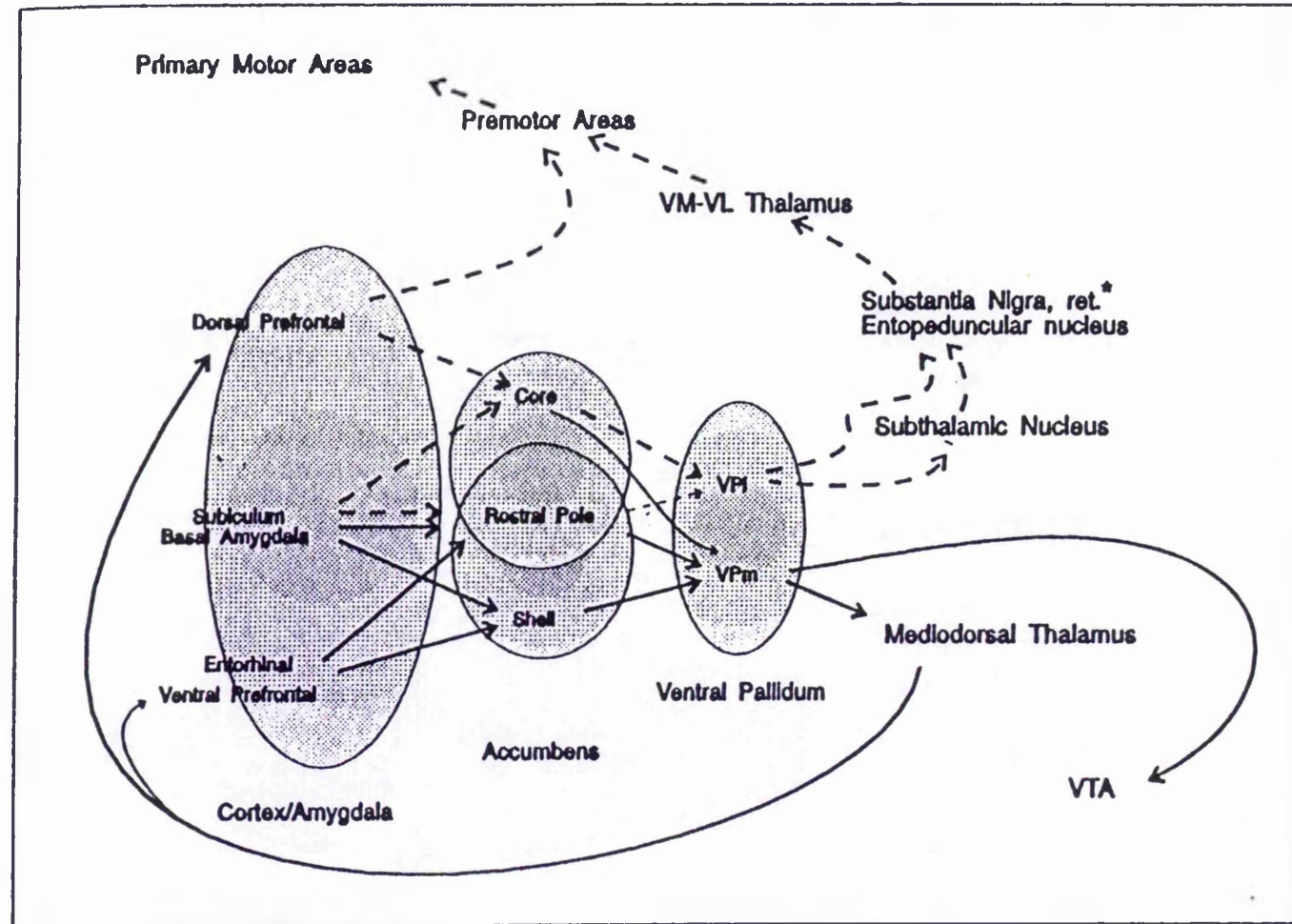
The core and shell of the NAcc also have separate efferent projection targets (Figure 3:1). Whereas the core projects into conventional basal ganglia circuitry, the shell terminates instead in limbic regions (Heimer *et al.*, 1991). Specifically, the core projects to the dorsolateral ventral pallidum, with limited additional connections with the GP and ventromedial region of the ventral pallidum. The core also projects to the medial portion of the EP and the adjoining region of LH, but projections from this region to the SNr are sparse. Part of the efferent outflow of the core is to the SNc and this provides an opportunity for the ascending A10 portion of the DA projection to influence activity in the A9 nigrostriatal pathway. The shell bypasses these "motor" structures to terminate instead in the ventromedial ventral pallidum, ventral parts of the bed nucleus of stria terminalis, lateral preoptic region, sublenticular substantia innominata and the entire length of the LH. The shell also innervates the VTA, retrorubral field (A8) and brainstem extrapyramidal area in the pons. In the rostral pole of the NAcc, where a distinction between core and shell cannot be made, connections are like those of both projection systems (Zahm and Heimer, 1993). While the efferents of the lateral rostral pole are reminiscent of core, those of the medial rostral pole are reminiscent of shell (Figure 3:2). Thus the NAcc as a whole is a neural structure related anatomically to the dorsal striatum and to the limbic system and it may therefore be in a crucial position for integrating motoric and limbic functions (Mogenson, 1987).

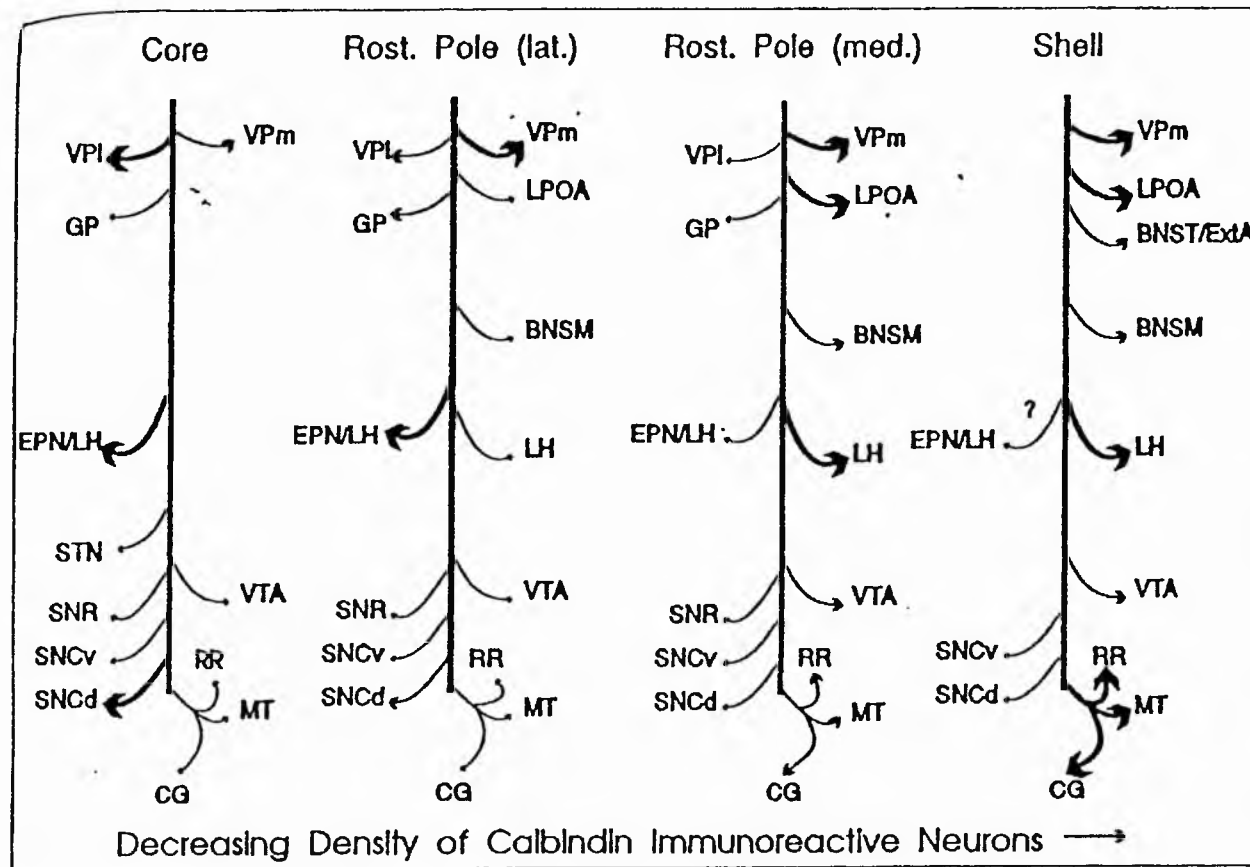
Figure 3:1

Flow diagram taken from Zahm and Brog, 1992 (Figure 5) illustrating the cortical origins and subcortical targets of the different subterritories of the nucleus accumbens. Note that the core connects predominantly with motor sites, while the shell is more closely linked to limbic structures.

Figure 3:2

Illustration taken from Zahm and Heimer, 1993 (Figure 8), summarising the differences and similarities between the efferent projections of the different subterritories of the nucleus accumbens.





Despite obvious connectional differences, strict boundaries between dorsal and ventral striatum are not actually considered to exist. It has instead been suggested that there are "transition zones" not only between CPu and NAcc, but also between the different zones of the NAcc (Zahm and Brog, 1992). In these transition zones between adjacent territories and subterritories there are known to be histochemically distinct cell groups which may have their own specific combination of connections (Voorn *et al.*, 1989). For instance, just as the rostral pole of the NAcc is a specialised combination of core and shell projections, it is conceivable that there are transition regions between dorsal and ventral striatum which are neither specifically dorsal nor specifically ventral in their connectivity. Indeed, it is unclear whether they even conform to the conventional patch-matrix organization associated with the striatum (see p. 47).

Compartmental organisation in the dorsal striatum

The dorsal striatum is composed of one principal neuronal type, the medium spiny neurone. These neurones have a medium-sized cell body, approximately 20-25 μm in diameter, from which radiate branched dendrites that are densely laden with spines (Kemp and Powell, 1971a). Their dendritic branches typically extend 150-250 μm in diameter and so adjacent neurones are often in receipt of common inputs. Cortical and thalamic excitatory inputs predominantly make asymmetric contact with the heads of the dendritic spines (Bouyer *et al.*, 1984) while dopaminergic inputs from the SNc and VTA make symmetric synaptic contact with the necks of dendritic spines and on the interspine dendritic shafts (Bouyer *et al.*, 1984; Freund *et al.*, 1984). In addition to this neuronal population, approximately 10% of striatal neurones are aspiny and intrinsic to the striatum. These include large cholinergic aspiny neurones (Bolam *et al.*, 1984) and medium aspiny neurones containing a diversity of neurotransmitters such as somatostatin and neuropeptide Y (DiFiglia and Aronin, 1982; Vincent and Johansson, 1983).

Although the CPU is cytoarchitecturally homogeneous, subpopulations of dorsal striatal neurones can be organized into separate functional compartments on the basis of their extrinsic connections. There are three general levels of compartmental organization which relate to the neostriatum. These are not mutually exclusive, but instead represent overlapping sets of subpopulations of striatal output neurones. First, there is the level of organization defined by the segregation of striatal output neurones into patch and matrix components (Bolam *et al.*, 1988; Gerfen, 1985); second, there is the separation of projections to the external segment of the GP and the SNr (Gerfen *et al.*, 1990); and third, there is the topography of separate cortical inputs.

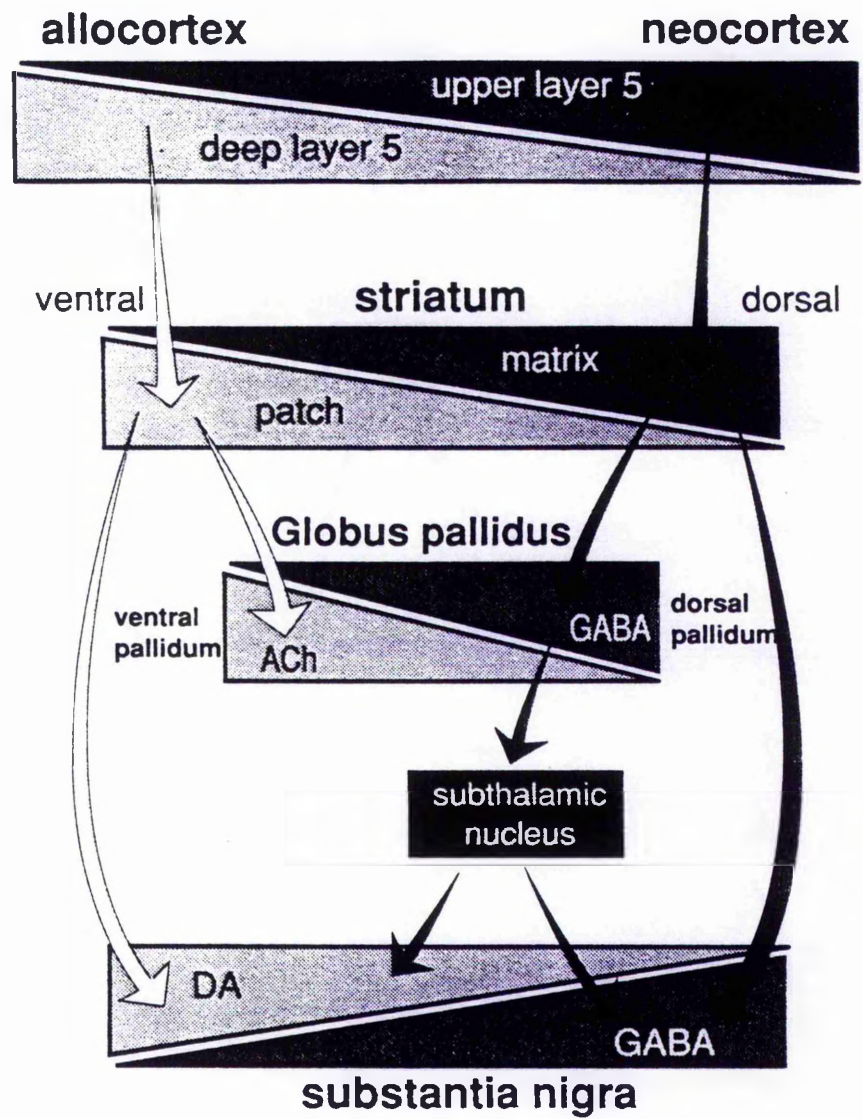
Patch and matrix. The patch and matrix compartments of the striatum are discriminated both on the basis of specific neurochemical markers and their neuronal connections. The patch component is defined by areas of dense μ -opiate receptor binding (Herkenham and Pert, 1981) and low AChE labelling: patches are also referred to as striosomes (Graybiel and Ragsdale, 1978). The matrix component is complementary to the patches and consists of neurones which are rich in calbindin and somatostatin (Gerfen, 1985; Gerfen *et al.*, 1985). As well as in the rat, these compartments have also been characterized in the cat (Jimenez-Castellanos and Graybiel, 1989) and primate (Gerfen *et al.*, 1985). Distribution of peptides such as enkephalin or substance P have been shown to be distributed in a heterogeneous manner which may or may not relate to the patch and matrix components (Gerfen, 1992). Numerous studies have demonstrated that the dendrites of patch and matrix medium spiny neurones remain restricted for the most part to the compartment of the parent neurone (Herkenham *et al.*, 1984; Gerfen, 1985; Bolam *et al.*, 1988; Kawaguchi *et al.*, 1989). This is particularly important as it suggests that inputs which are confined specifically to patch or matrix compartments will only affect patch or matrix output neurones respectively.

Although the neurochemical markers of the patch and matrix display a consistent complementary pattern in the rat throughout the majority of both the dorsal and ventral striatum, within some regions of the NAcc they are not as distinct (Voorn *et al.*, 1989). In the caudomedial septal pole of the shell, the core/shell border and the rostral pole, there are regions which exhibit high μ -opiate receptor binding but weak AChE and enkephalin activity. These regions, called cell clusters, comprise very tightly packed regions of cells (Herkenham *et al.*, 1984) and are connectionally distinct from the patch and matrix. Two enkephalin rich zones have also been described (Voorn *et al.*, 1989) which may or may not relate to patch and matrix (Zahm and Brog, 1992). The first of these, in the division between the core and the ventromedial CPu, is rich in substance P and has weak calbindin immunoreactivity suggesting that it may in fact be related to the patch (Gerfen *et al.*, 1987). The second enkephalin-rich zone is located in the region between the dorsolateral rostral NAcc and the ventrolateral CPu. This calbindin rich region may relate to a rostrolateral specialisation of the matrix compartment (Gerfen *et al.*, 1985).

Patch-matrix compartmental organization is likely to be related to the lamination of the cortex and as such cortical inputs to patch and matrix may provide the clearest indicator of the functional significance of this compartmentalization. For example, the use of the anterograde tracer PHA-L has demonstrated differences in the projections of the sublaminae of layer 5 to the striatum (Gerfen and Sawchenko, 1984) (Figure 3:3). The cortical inputs to the patch were shown to originate from deep layer 5 of the cortex, while those inputs to the matrix arose from upper layer 5 and the supragranular layers. Similarly, although all cortical layers appear to send projections to both of these striatal compartments, their relative input varies such that periallocortical areas send a dense input to the patch while neocortical areas do not (Gerfen, 1989). Furthermore, tracer studies in primates have demonstrated that injections into neocortical areas label neurones which are primarily associated

Figure 3:3

Illustration taken from Gerfen, 1992 (Figure 3) representing some of the differences in the cortical inputs and basal ganglia outputs of the patch and matrix components of the striatum.



with the matrix component (Goldman-Rakic, 1982). These tracer studies support the conjecture that the patch compartment has strong links with limbic function while the matrix might be more closely tied to sensorimotor information.

Further endorsement of this type of compartmentalisation comes from evidence of functional relationships between striatal patches and the amygdala (Ragsdale and Graybiel, 1988) or the patches and the prelimbic cortex (Gerfen, 1984; Donoghue and Herkenham, 1986) in addition to links between sensorimotor cortex and the striatal matrices (Gerfen, 1984; Donoghue and Herkenham, 1986). The patch compartment is avoided by projections from the midline and intralaminar thalamic nuclei (Herkenham and Pert, 1981) and the primate putamen contains very little of the patch compartment (Goldman-Rakic, 1982). Although in the rat there are known to be both prelimbic and sensorimotor cortical inputs to each compartment (Gerfen, 1989), their relative contribution varies markedly. Retrograde tracing studies in rats have demonstrated that the limbic/motor dichotomy of patch and matrix is also relevant to the outputs of the striatum. Although both the patch and matrix send inputs to the SN, patch neurones innervate the dopaminergic neurones in the ventral tier of SNc and in the SNr islands, while the matrix neurones project to the location of GABAergic neurones in the SNr (Gerfen, 1984; Gerfen, 1985; Gerfen *et al.*, 1985). In this way, matrix neurones can be seen directly to influence motor output through the SNr while the patches have indirect influence on successive motor outputs through integration with nigrostriatal feedback circuitry. Hence, classification of striatal circuitry into patch and matrix compartments can not only be seen to clarify separate cortical inputs to the striatum, but also has the functional utility of delineating a "limbic-oriented" versus "motor-oriented" form of striatal segregation.

Striatopallidal and striatonigral projections. In addition to the categorization of medium spiny neurones into patch and matrix, the dorsal striatum can be arranged

in terms of its output pathways to the GP, EP (internal segment of the GP in primates) and SNr. There is extensive collateralisation to each of these target nuclei, but the relative extent of terminal arborization in a particular target area suggests definition of two separate major output pathways (Kawaguchi *et al.*, 1990). In this study striatopallidal neurones sent axons to the GP which did not generally appear to collateralise beyond this nucleus, while striatonigral neurones not only projected to the SNr/EP and arborized extensively there, but also sent axonal collaterals to the GP without extensive terminal ramifications. It has been estimated that these separate channels of output from the striatum comprise approximately equal numbers of neurones (Gerfen and Young, 1988).

Although the neurones of the striatonigral and striatopallidal pathways have similar morphology and neurochemistry, they can be differentiated on the basis of neuropeptide and dopamine receptor subtype expression. For instance, *in situ* hybridisation histochemistry has determined that the majority of striatonigral neurones express substance P, dynorphin and the D₁ dopamine receptor while the majority of striatopallidal neurones express the peptide enkephalin and the D₂ dopamine receptor (Gerfen and Young, 1988; Gerfen *et al.*, 1990). It should be emphasised however that these data did not imply exclusive expression of D₁ and D₂ receptors in striatonigral and striatopallidal neurones respectively. In fact at least 15-20% of striatal output neurones which expressed neurokinin B also expressed both D₁ and D₂ subtypes of dopamine receptor (Surmeier *et al.*, 1993).

Physiological activity in the two channels differentially modulate the GABAergic neurones in the SNr. On the one hand the striatonigral pathway provides a direct GABAergic inhibitory input to the SNr, while the striatopallidal pathway on the other provides an indirect connection between the striatum and the SNr: striatopallidal neurones inhibit the GP through GABAergic transmission and in turn send further GABAergic neurones to the SNr and STn (Smith *et al.*, 1990). The

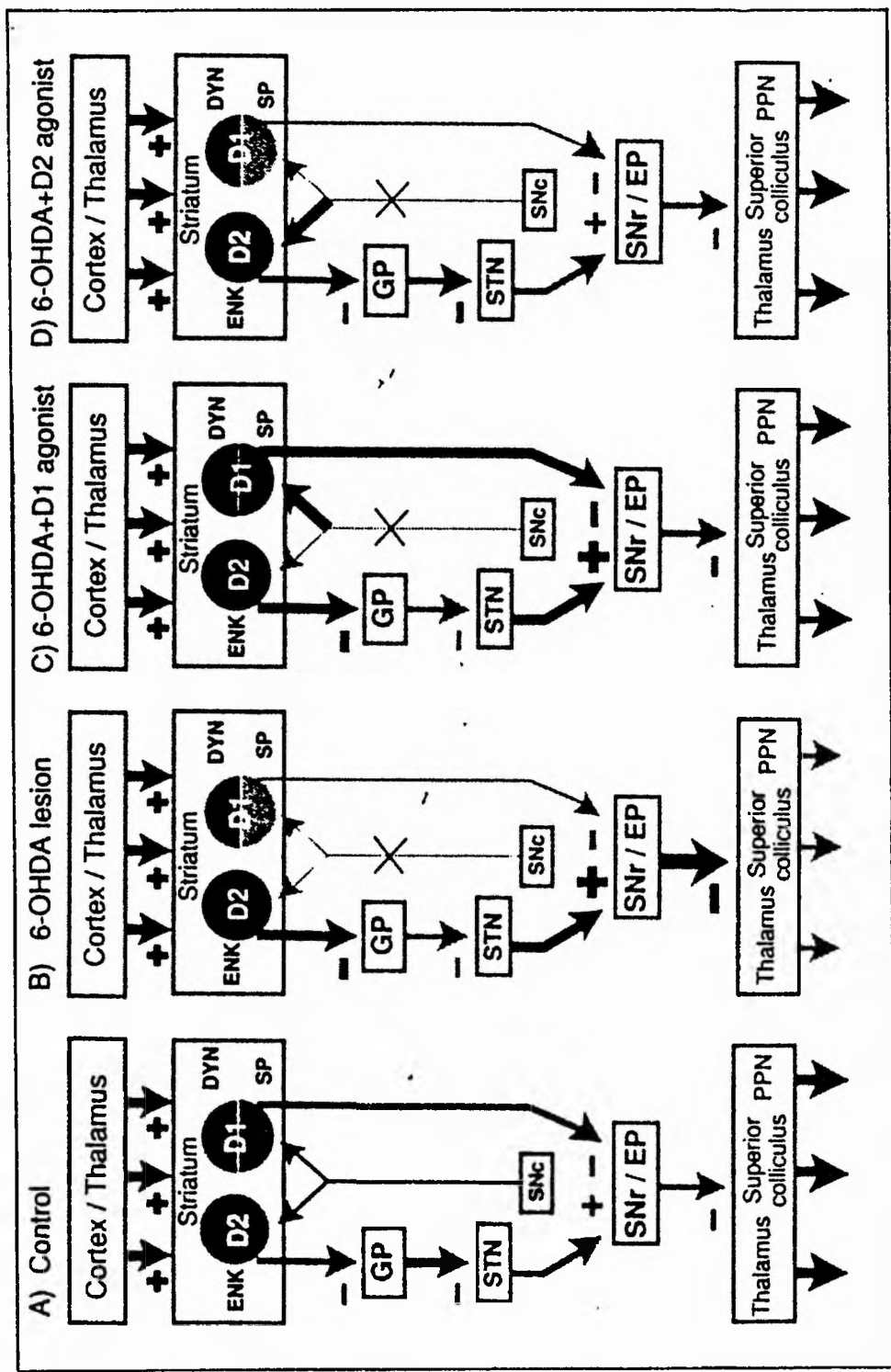
STn provides an excitatory input to the SNr (Kita and Kitai, 1987). The activity of the SNr is therefore controlled by the relative responsiveness of these separate striatal output channels to their cortical inputs and balanced output must be maintained for the generation of normal movement. The relative positions of striatal and pallidal terminals in SNr (Smith and Bolam, 1991; Von Krosigk *et al.*, 1992) are also likely to be crucial to this balance.

Lesions which deplete levels of dopamine in the striatum result in decreased levels of dynorphin and substance P mRNA expression in striatonigral neurones and increased enkephalin mRNA expression in striatopallidal neurones (Gerfen *et al.*, 1990). These changes in turn produce an imbalance in the signals reaching the SNr and induce an increase in the activity of nigral output neurones as indicated in Figure 3:4. Treatment with D₁ or D₂ agonists restores this balance and returns inhibitory signals elicited from the SNr to normal. For instance, treatment with the specific D₁ agonist SKF-38393 reverses the lesion-induced reduction in substance P and significantly increased dynorphin mRNA expression in striatonigral neurones without altering the lesion-induced increase in enkephalin mRNA expression in the striatopallidal pathway (Gerfen *et al.*, 1990). In an analogous way post-lesion treatment with the D₂ agonist quinpirole reverses the increased enkephalin mRNA expression in the striatopallidal neurones but has no effect on the striatonigral pathway (Gerfen *et al.*, 1990). These data suggest that dopamine oppositely modulates gene expression in striatonigral and striatopallidal neurones via the respective expression of D₁ and D₂ receptor subtypes.

These changes in gene expression may parallel physiological changes which occur in these neurones in the striatal DA deafferentation which occurs in Parkinson's disease. In primates made parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 2-deoxyglucose (2-DG) autoradiography revealed a profound increase in 2-DG uptake in the striatopallidal pathway and a consequent

Figure 3:4

Flow diagram taken from Gerfen *et al.*, 1990 (Figure 4) illustrating the relative activities of separate striatal output pathways following dopamine deafferentation in the striatum and subsequent treatment with specific receptor agonists (measured by 2-deoxyglucose: Trugman and Wooten, 1987).

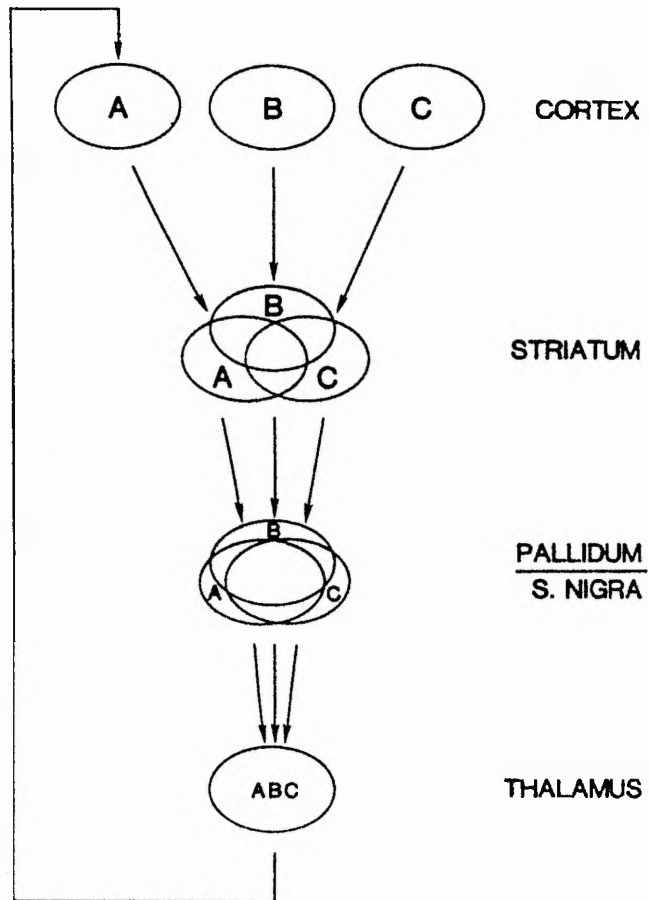


decrease in its uptake in the external pallido-STn pathway (Mitchell *et al.*, 1989). Elevation in striatopallidal activity following nigrostriatal DA depletion would account for the bradykinesia associated with this disease and will be discussed more fully later.

Cortical inputs to the striatum: the organization of segregated loops. In the 1970s the dorsal striatum was characterized as a relay station for funneling a diversity of cortical inputs via the ventrolateral thalamus to the motor cortex, integrating it at the level of the striatum with sensory information related to the initiation and control of movement (Evarts and Thach, 1969; Kemp and Powell, 1971b). This view of one general transmission system was subsequently broken down into two functionally and anatomically segregated loops through the basal ganglia (DeLong and Georgopoulos, 1981). The "motor loop", projecting via the putamen, linked the sensorimotor cortex to the ventroanterior-ventrolateral thalamus and premotor areas; the "complex loop" was thought to pass through the caudate, linking association cortex to the ventrolateral thalamus and areas of the prefrontal cortex. This scheme has now been further revised in favour of one involving five basal ganglia - thalamocortical circuits (Alexander *et al.*, 1986). There appear to be at least 4 circuits - oculomotor, dorsolateral prefrontal, lateral orbitofrontal and anterior cingulate - organized in parallel to the motor circuit. Each is thought to receive multiple partially overlapping corticostriate inputs which are progressively integrated *en route* for specific portions of the thalamus before projecting back to a discrete cortical region. Each circuit receives its striatal inputs from functionally related cortical regions (Figure 3:5). For instance, the "motor" circuit receives inputs from at least 4 cortical regions implicated in the control of limb and orofacial movements (supplementary motor area, arcuate premotor area, motor cortex and somatosensory cortex), while the "oculomotor" circuit receives inputs from 3 interconnected areas (frontal eye fields, dorsolateral prefrontal cortex and posterior parietal cortex) associated in the control of eye movements.

Figure 3:5

Template of a basal ganglia-thalamocortical loop taken from Alexander *et al.*, 1986 (Figure 2), depicting the proposed linking of information arising from anatomically disparate, but functionally related cortical sites. After converging, information from the thalamus is funnelled back to one of the original cortical inputs.



Striatal dopamine and behaviour

The majority of reported studies of DA function have involved the use of lesions of particular DA-containing fibre systems followed by testing for behavioural deficits. Others have approached the role of DA in normal, behaving animals through (i) the observation of the effects of systemic DAergic drugs on behaviour, (ii) the effects of iontophoretically applied DA, or microinjections of dopaminergic drugs on behaviour and (iii) the use of electrophysiological studies, which investigate changes in firing rate of DA neurones to particular stimuli.

General behavioural dissociation of the dorsal and ventral striatum

The separable functions of the NAcc and CPu were clearly demonstrated by Kelly and colleagues, who showed that DA depletion in either structure can antagonize different behavioural effects of *d*-amphetamine (AMPH) (Kelly *et al.*, 1975). Specifically, the locomotor effects of AMPH were attenuated by DA depletion in the NAcc while the stereotyped head movements and sniffing behaviour were diminished by lesions in the CPu. The opposite constellation of effects occurred following apomorphine (APO) injection and this was thought to indicate a development of supersensitivity of postsynaptic DA receptors following presynaptic terminal destruction. Suggestions that the locomotor effects in the NAcc lesion group could be attributed to NA depletion, were subsequently ruled out by blocking the NA depletion following 6-OHDA by pretreatment with desipramine (Kelly and Iversen, 1976).

Mittleman and his colleagues have also recently compared dorsal and ventral striatal function. They examined deficits in schedule-induced polydipsia (SIP) following 6-OHDA-induced DA depletion in CPu or NAcc, or aspiration lesions of the neocortex or hippocampus (Mittleman *et al.*, 1990). Broadly speaking there were 2 distinct behavioural impairments following these lesions which provided a double dissociation between the effects of DA depletion on different parts of the

striatum on motivational and motor functions. Rats with neocortical and CPu lesions reduced their water intake during SIP but increased their licking activity. This decreased "lick efficiency" suggests a motor impairment in the use of tongue and mouth movements. Conversely, hippocampal- and NAcc-lesioned rats showed decreased licking and a concomitant decrease in water consumption which have been attributed to motivational types of deficits.

Caudate nucleus and putamen

The early work investigating the functions of the nigrostriatal DA system was carried out as part of investigations of the lateral hypothalamic syndrome (LH syndrome). Anand and Brobeck (1951) demonstrated that bilateral electrolytic lesion of rat lateral hypothalamus (LH) produced a syndrome of aphagia, adipsia and akinesia as well as a sensorimotor dysfunction. However, as the nigrostriatal pathway courses through the LH region without synapsing there, it was acknowledged that LH syndrome could in fact be caused by nigrostriatal fibre damage. This was investigated using bilateral injections of the catecholamine-selective neurotoxin 6-hydroxydopamine (6-OHDA) into the LH and various other sites along the DAergic fibre pathway (Ungerstedt, 1971). Where these injections induced bilateral >90% depletions of DA from the dorsal striatum, most of the symptoms of classic LH syndrome were reproduced. Less complete lesions, or those where damage was predominantly unilateral, induced catalepsy, difficulties in initiating movement and transient postural asymmetries, although the use of DAergic drugs demonstrated that this recovery of posture was due to synaptic compensation. Treatment with the DA-releasing agent *d*-amphetamine induced ipsilateral rotation due to larger DA efflux on the intact side and the crossed nature of the pathways from the striatum. Conversely, the direct DA agonist apomorphine induced contralateral turning due to possibly due to increased sensitivity of DA receptors on the postsynaptic membrane following DA denervation on the lesioned side (see Ungerstedt, 1971).

The general sensorimotor neglect observed following electrolytic lesions of the LH was also present following unilateral nigrostriatal DA depletion. This neglect syndrome has been measured in all sensory domains following such lesions. For instance it was ascertained whether the feeling of touch remained by applying a von Frey hair to the body surface separately on each side and whether the sensation of smell was affected by observing the reactions to a strong smell such as ammonia (Marshall and Teitelbaum, 1974). Rats were unable to orient towards contralateral stimuli in this test and it was proposed that this deficit in orientation was due to contralateral sensory inattention and lack of sensorimotor integration following striatal DA disruption. Subsequently, these terms have been used in a vague attempt to describe the nature of the neglect while the functions of nigrostriatal DA remained unclear. The problem with many tests of neglect is that they do not dissociate between problems with the detection of contralateral stimuli and problems with integration of correct stimulus detection into the appropriate orientating and motor output responses. For instance, contralateral neglect could occur as a result of a sensory or attentional impairment, a failure to initiate a response to contralateral space, or an inability to produce contralateral responses. As a result of this, many subsequent studies have attempted to separate simple deficits in "sensory" and "motor" function in rats with nigrostriatal DA lesions.

For example, Turner (1973) used a paradigm which involved training rats to make head turns either towards or away from the side of the lesion in response to footshocks administered either ipsilaterally or contralaterally to the lesion. The only group which had difficulty in learning the head turn escape response was the one required to make a contralateral head turn in response to a contralateral stimulus. Turner concluded that the main deficit was neither simply sensory nor simply motor and suggested that the deficit arose from an impairment of "intra-hemispheric sensorimotor integration". In a comparable choice paradigm where responding was appetitively reinforced, Hoyman and colleagues (Hoyman *et al.*,

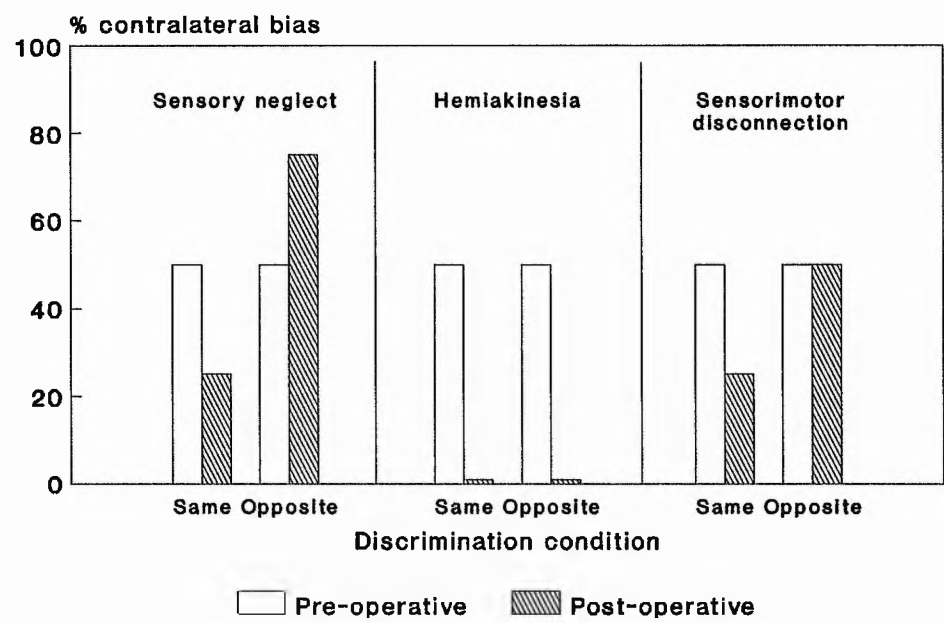
1979) found that unilateral electrolytic lesions of the LH led to consistent deficits in responding to contralateral tactile stimuli, irrespective of whether the rats were required to respond towards or away from them. Therefore, it seemed that the deficit was in making responses to contralateral space, or "hemiakinesia" as termed by Milner (1987).

More recently, Carli and colleagues (Carli *et al.*, 1985) attempted to resolve the confusion further by separating sensory and motor factors in the neglect syndrome using a novel paradigm. Rats were trained on an unpredictable schedule to detect brief flashes of light to either side of the head and the speed and accuracy of responses collected by computer. In one condition rats were trained to respond in the same place as the light while in the alternative condition they were trained to respond in the location away from the side of the light. It was evident that this task would be able to dissociate between each of the major hypotheses of neglect and the predictions for response patterns in the different types of neglect are summarised in Figure 3:6. Following training, injections of 6-OHDA were made into the mid-ventral head of the caudate, which depleted DA in both anterior and posterior caudate by 70-90% without inducing a significant decrease in accumbens DA levels. The separation of stimulus and response contingencies which was achieved in this task showed that rats with DA depletion from the dorsal striatum on one side were impaired whenever they had to respond to contralateral space, regardless of whether they were responding to a stimulus there or not. This result is consistent with that from Hoyman and colleagues (1979) and further suggests that the neglect syndrome obtained following nigrostriatal DA loss is a form of hemiakinesia. Reaction time measures revealed additionally that the problems were in the initiation rather than the completion of the movement. Consistent with these data are results from a separate group of lesioned rats who had been trained to acknowledge a light-flash by pushing a panel behind them. This group were not impaired at detecting a contralateral stimulus and demonstrated no enhancement of

Figure 3:6

Bar graph redrawn from Carli *et al.*, 1989 (Figure 1) illustrating the likely outcome in their discrimination task from each of the suggested hypotheses of neglect. After extensive training, the pre-operative contralateral bias will be approximately 50%. Post-operatively, the sensory neglect hypothesis asserts that rats will always respond correctly on the ipsilateral side, but only guess on the contralateral side; the hemiakinesia hypothesis implies a failure to respond contralaterally regardless of the side of stimulus presentation; and the disconnection hypothesis dictates that the rat is only impaired when making contralateral responses to contralateral stimuli.

Figure 3:6



sensory attention towards the ipsilateral side. Together these studies suggest that the nature of the impairment is in the initiation of response-related actions to contralateral space.

These findings have subsequently been extended in several ways (Carli *et al.*, 1989). Once again visual stimuli were used and reaction times measured in computer-controlled experiments. Four groups of rats were given specific neurotoxic lesions of forebrain DA terminals within the striatum: 6-OHDA was infused unilaterally either to NAcc or CPu, or was infused to NAcc and CPu on the same or on opposite sides. In a replication of the previous study, the results showed that all rats, except those receiving only intra-accumbens 6-OHDA, exhibited a significant bias in responding towards the side of the lesion and this correlated with DA depletion in the head of the caudate nucleus. A more detailed analysis of the behavioural evidence clearly demonstrated that unilateral striatal DA depletion did not produce sensory loss or sensory inattention. Firstly, the pattern of results for this type of neglect differed from those for the sensory hypothesis as shown in Figure 3:6. Secondly, it is likely that those rats showing an ipsilateral response bias were still able to determine the side of the stimulus presentation, because the reaction times for correct and incorrect ipsilateral responses were significantly different and were independent of the type of dissociation being performed. This also argues against a lesion-induced stereotyped motor tendency because a uniformity of reaction times would have been expected if this were the case. As in the previous study, the biased groups were slower to initiate, but not to complete, contralateral responses. This is further suggestive of an output type of neglect related to hemiakinesia and which may be best described as an impaired "attention to action" (Carli *et al.*, 1989).

These impairments seen in the bias of responding towards one side and those in initiation or reaction time (Carli *et al.*, 1985; Carli *et al.*, 1989) are consistent with

other evidence that striato-pallido-thalamo-cortical circuitry participates in the preparation for action (Goldberg, 1985). It is also important to integrate these results with information of how nigrostriatal DA neurones influence striatal output neurones. For example, it is known that 35% of the terminals of nigrostriatal DA neurones synapse on to dendritic shafts while 55-60% connect with dendritic spines; 70% of these synapses are with striatonigral neurones (Freund *et al.*, 1984). Therefore nigrostriatal DA directly influences at least one of the major output pathways from the dorsal striatum, the direct route to the SNr. The inputs to the dendritic shafts are most likely to influence the extent to which information from more distal sites on the dendrite or from the spines reaches the cell body. The inputs to the dendritic spines always occur in conjunction with an input from a non-TOH immunoreactive neurone and analysis of degenerating boutons in the striatum following cortical lesions suggests that this non-TOH-immunoreactive input originates from the cortex. In most cases the TOH-immunoreactive bouton is closer to the dendritic shaft and it is therefore in a position to control directly the impact of neuronal firing arising from the other, probably cortical, input.

Although it is controversial whether the actions of DA are excitatory or inhibitory (Siggins, 1978; Herrling and Hull, 1980) the predominant action is a slow depolarisation followed by a decrease in spontaneous firing rate (Herrling and Hull, 1980; Rolls *et al.*, 1984). Rolls and colleagues were able to record from neurones in the putamen which normally fired in response to mouth movements and then monitor the firing of these neurones during iontophoretic application of DA (Rolls *et al.*, 1984). It was demonstrated that DA increased the signal-to-noise ratio of striatal neurones. It has therefore been suggested that DA might enhance the transmission of information through the striatum during active neuronal firing by ensuring that the spontaneous firing rate (neuronal noise) is minimal (Rolls *et al.*, 1984).

DA in the dorsal striatum is clearly in an appropriate anatomical location to have a direct modulatory effect on other inputs to striatonigral neurones. Changes in motor output would be partly controlled by altering the relative significance of separate cortical inputs by means of DAergic inputs to the CPu. For instance, a tonic cortical activation could be manipulated by DA at some of the dendritic spines to produce a variable, patterned output. This might imply that the ascending nigrostriatal DA projection does not participate directly in those neural processes by which actions are selected, but rather that it exerts a modulatory influence that is expressed when an action has been selected and is being prepared for execution. This sort of interpretation of DA priming certain motor outputs is consistent with the ipsilateral response bias and the deficits in contralateral initiation times observed following nigrostriatal DA depletion in the Carli studies (Carli *et al.*, 1985; Carli *et al.*, 1989). It also complements evidence that regional differences in corticostriatal connectivity account for behavioural heterogeneity in the striatum and implies that the behavioural disturbances seen following lesions of the entire CPu can be attributed to the disruption of separable corticostriatal channels of information (Alexander *et al.*, 1986).

Separable functions of dorsal striatal subregions. In the rat the medial striatum receives projections mainly from the medial prefrontal cortex (Beckstead, 1979) and so, like this region of cortex, it selectively influences spatial learning tasks (Divac, 1972; Divac *et al.*, 1978). In contrast, the lateral striatum receives projections mainly from regions of the dorsolateral frontoparietal cortex (Wise and Jones, 1977) that are known, through electrophysiological mapping studies, to be related to sensorimotor tasks (Donoghue and Wise, 1982). This projection may have a role in segmental motor control as damage to somatic sensorimotor cortex or to the lateral striatum produces impairments of tongue protusion and forelimb reaching (Castro, 1972a; Castro, 1972b; Pisa, 1988). Similarly, rats with caudate lesions were impaired in their control of tongue and mouth movements in the

acquisition of SIP (Mittleman *et al.*, 1990). The relative contributions of the medial and lateral CPu in the task used by Carli and colleagues have also been examined by comparing the effects of unilateral ibotenate lesions to these discrete regions in the rat striatum (Brown and Robbins, 1989). Lateral striatal lesions produced a strong response bias towards the side of the lesion but left the latency for the initiation of responses to the visual cues unchanged, while the medial striatal lesions resulted in a smaller degree of spatial response bias but a significant impairment of contralateral response initiation.

Investigations of lever-pressing where only those responses separated by a delay are reinforced (differential reinforcement of low rates: DRL) have further highlighted functionally separate regions of lateral and medial CPu. For instance, bilateral lesions of the ventrolateral, but not anteromedial, caudate-putamen with kainic acid or 6-OHDA disrupted acquisition of DRL-20 sec operant schedule (Dunnett and Iversen, 1982). The pattern of operant responding in this group suggested that they were deficient in response sequencing or switching. These data also complement the impairments in tongue and forelimb use which were described following bilateral lateral striatal lesions (Pisa, 1988) and together with this study suggest that the ventrolateral lesion group might have specific difficulty in sequencing or switching between the alternative pellet-retrieving and lever-pressing responses.

Investigations of oral motor and ingestive behaviour have revealed further differences between subregions of the lateral CPu. Infusions of AMPH into a ventrolateral region of the CPu, but not a dorsolateral region induced increased levels of feeding behaviour as measured not only by increased food intake, but also by increased number of feeding bouts and a decreased latency to feed (Kelley *et al.*, 1989). Many of the rats injected in this region also displayed an intense stereotyped biting of their forepaws for between 10 and 30 min after an injection of 5-20 μ g

AMPH (Kelley *et al.*, 1988; Kelley *et al.*, 1989). This intense form of biting behaviour can only be elicited following concurrent stimulation of both D₁ and D₂ receptors in the ventrolateral CPu (Delfs and Kelley, 1990). Stimulation of D₁ alone with SKF-38393 did not induce any change in oral behaviour, while D₂ receptor stimulation by quinpirole increased licking, head-down sniffing and mouth movements.

Although traditionally thought of as a substrate for motor function, the nigrostriatal system has also been shown through animal studies to be involved in cognitive function. CPu lesions produce impairments in passive avoidance learning and retention (Sanberg *et al.*, 1978) and appetitively motivated T-maze spatial alternation learning (Pisa *et al.*, 1980). The use of spatial cues in solving the Morris water maze was also disrupted by lesions in the CPu (Whishaw *et al.*, 1987). It may be that in order to understand these deficits the distinction between egocentric (position relative to the body) and allocentric (position invariant in space) memory may be critical. For example, Cook and Kesner (1988) found that rats with caudate lesions show deficits on radial-arm test paradigms requiring egocentric but not allocentric memory. Such cognitive impairments have important implications for diseases of the basal ganglia, notably Parkinson's disease. Post-mortem studies reveal at least 80% depletion in the parkinsonian putamen (implicated in the motor symptoms of the disease), but much less in the caudate (Agid *et al.*, 1987) which is seen as playing a role in the pattern of cognitive impairment found as the disease progresses.

Nucleus accumbens

Locomotor activity. In addition to the previously described study showing that DA depletion in NAcc attenuated locomotor activity in response to AMPH and APO (Kelly *et al.*, 1975), more recent studies have demonstrated that if tested 7 (but not 28) days after surgery, 6-OHDA lesions of the NAcc decrease spontaneous

locomotor activity (Koob *et al.*, 1978; Winn and Robbins, 1985). It had been suggested that the initial lesion-induced hypoactivity (Koob *et al.*, 1978) might reflect a deficit in exploratory behaviour, as rats with mesolimbic DA depletion induced by 6-OHDA infusions into the anterolateral hypothalamus were less active in open field tasks and impaired in their investigation of novel stimuli (Fink and Smith, 1979). However, it has subsequently been demonstrated that mesolimbic DA depletions achieved by this method also markedly decreased levels of DA in the CPu and so the behavioural results from this study cannot be attributed solely to loss of mesolimbic DA (Winn and Robbins, 1985). Measurement of novelty carried out in the exploration choice box (Sahakian *et al.*, 1977) following lesions of NAcc DA terminals demonstrated that at 7 days post-surgery, rats exhibited a non-significant trend towards reduced novelty preference (Robbins and Everitt, 1982), while at 28 days post-operatively there were no deficits on measures of exploration (Winn and Robbins, 1985).

Schedule-induced behaviours. When food-deprived rats are exposed to fixed-interval schedules of reinforcement they develop periods of excessive drinking (Falk, 1961) which cannot be explained either in terms of a physiological deficit or as a result of adventitious temporal pairing between drinking and the delivery of food (Wetherington, 1982). This adjunctive behaviour has been termed schedule-induced polydipsia (SIP), is considered to be a form of displacement behaviour (Falk, 1971). 6-OHDA lesions of the NAcc impair acquisition of SIP (Robbins and Koob, 1980) through a decrease in schedule-induced licking and water consumption in the phase where maximal licking normally occurs (Mittleman *et al.*, 1990). These deficits do not result from primary motivational factors, as rats with 6-OHDA lesions of the NAcc exhibit no general impairments in regulatory ingestive behaviour (Robbins and Koob, 1980). The main hypotheses of SIP acquisition emphasise motivational factors. For instance, some authors have related SIP to the incentive motivational properties of the delivered food pellets (Killeen *et*

al., 1978), while others have suggested possible functions of SIP in coping with elevated levels of arousal between delivery of food pellets (Brett and Levine, 1979).

Conditioned reinforcement. The conditioned reinforcement paradigm has further demonstrated a role for mesolimbic DA in mediating reward-related behaviours. Thirsty or food-deprived rats are first trained to associate a compound light/sound stimulus (CR) with food or water presentation. Then, in the test phase, responding on one lever (CR lever) produces the light and sound stimuli without food/water presentation, whereas responding on the other (NCR lever) has no effect. The efficacy of the conditioned reinforcer (light/sound) is measured by the extent to which it supports acquisition of the responding on the CR lever in the absence of the primary reward (Mackintosh, 1974). Dose-dependent effects on the CR lever have also been obtained following systemic administration of psychomotor stimulants such as pipradol (Robbins, 1978), AMPH and some analogues of cocaine (Robbins *et al.*, 1983), but not following systemic administration of methylphenidate (also a psychomotor stimulant) (Robbins, 1978) or following drugs outside this class such as morphine, α -flupenthixol or chlordiazepoxide (Robbins *et al.*, 1983). Infusions of AMPH into the NAcc but not CPu produces dose-dependent increases in responding on the CR lever, but no significant change on the NCR lever (Taylor and Robbins, 1984). This response can also be obtained following intra-NAcc (but not intra-CPu) DA administration and is not found following random pairings of the primary reinforcer with the compound stimulus (Cador *et al.*, 1991). 6-OHDA-induced depletions of DA in the NAcc blocked increases in responding on either lever following intra-accumbens AMPH, while rats with DA depletion of the posterior caudate showed increases in responding on the CR lever similar in magnitude to those of sham-lesioned groups (Taylor and Robbins, 1986). Although these lesions also produced significant loss of noradrenaline (NA) from the NAcc, it has subsequently been demonstrated that 6-

OHDA lesions of the dorsal noradrenergic bundle, which depleted NA levels in the NAcc by 90% without affecting DA levels there, did not affect the increased responding for CR (Cador *et al.*, 1991). These results indicate that enhanced responding for conditioned reinforcement observed following administration of psychomotor stimulants is critically dependent on DA activation in the ventral, rather than dorsal, striatum. DA-dependent mechanisms there are likely to interact with afferent projections from limbic structures such as the amygdala which is thought to have a direct role in associative learning processes and has been implied to have a role in responding for CR following NAcc AMPH (Cador *et al.*, 1989).

Conditioned place preference. In the conditioned place preference (CPP) paradigm, rats are placed initially in a start box and allowed freely to explore two compartments which they can enter through separate openings from the start box. The compartments are different colours (for instance black vs. white), may have different floor coverings (for instance sawdust vs. grid) and can be swabbed down such that they are distinguishable by smell (Everitt *et al.*, 1991; McAlonan *et al.*, 1993). Rats are then exposed to a primary reward (or an aversive stimulus) in one compartment and saline in the alternative compartment and this pairing can occur over one or several trials. In the test phase, they are again given free access to each compartment and time spent / measures of exploration in each compartment recorded. A change in preference for a particular compartment, as measured by a change in the time spent there before and after incentive pairing, suggests that an association between incentive and environment has been formed. As such, a CPP is formed if there is an increase in time spent in a compartment and development of a CPP is taken to imply an association between that environment and reward.

Microinjection of AMPH into the NAcc, but not the CPu, induces a CPP (Carr and White, 1983; Carr and White, 1986). It has also been demonstrated that microinjection of either the specific D₁ agonist SKF38393 or the D₂ agonist

quinpirole into the NAcc can induce strong preferences (White NM *et al.*, 1991). The ventral striatopallidal system has been implicated in formation of such preferences, as lesions of anterior or posterior ventral pallidum significantly attenuated CPP (McAlonan *et al.*, 1993).

Self-administration. Animals are able to learn to perform an operant response to produce direct intravenous or intracranial injection of certain compounds (Koob, 1992). Decreases in the concentration or increases in the interval between injections of a self-administered compound such as cocaine both lead to increases in rates of responding. Neurochemical studies using *in vivo* microdialysis have confirmed that DA efflux is increased in the NAcc during i.v. self-administration of cocaine. Rats which have been trained to self-administer cocaine in this way show a gradual increase in DA levels in the NAcc which simulates the pattern of self-administration (Hurd *et al.*, 1989).

Similarly, rats which had learned to self-stimulate electrically in the VTA were demonstrated to increase their utilization and turnover of DA (as measured by post-mortem analyses of ratios of DOPAC and HVA to DA) in the NAcc (Fibiger *et al.*, 1987). More recently, measurement of DA efflux in the NAcc induced by electrical self-stimulation of the VTA has been measured using *in vivo* voltammetry (Phillips *et al.*, 1989). Increases in DA release paralleled closely the increases in self-stimulation current and fluctuations in DA efflux appeared to correspond to the rate of bar-pressing. Systemic administration of either cocaine or the selective DA uptake blocker GBR 12909 also enhanced self-stimulation rates and increased the DA oxidation current in the NAcc.

DA receptors in the NAcc are also implicated in at least some of the rewarding aspects of ethanol and opiate self-administration. For instance, oral ethanol self-administration is diminished by DA receptor agonists injected into the NAcc

(Rassnick *et al.*, 1993) and opiates can increase DA release in the NAcc as measured by *in vivo* microdialysis (Di Chiara and Imperato, 1988). However, there is also evidence for DA-independent reward in cases both of ethanol and opiate self-administration (Koob, 1992).

Aversive tasks. Although a large part of the literature concerning the NAcc focusses on reward-related tasks, there is also considerable evidence to suggest that DAergic mechanisms in this structure are critically involved in responses to aversive situations. For instance, mild tail-pinch pressure for 8 min increased DOPAC levels, measured by *in vivo* voltammetry, in the rat NAcc by 70% for more than 2 h (D'Angio *et al.*, 1987). Similarly, intermittent bursts of tail-shock (1 mA intensity) increased DA levels, measured by *in vivo* microdialysis, in the rat NAcc by 39% (Abercrombie *et al.*, 1989). Increases in DOPAC were not observed in the prefrontal cortex in the former study (D'Angio *et al.*, 1987), but DA was increased there in the latter study (Abercrombie *et al.*, 1989).

The role of the NAcc in active avoidance behaviour has also been investigated. Many studies have implicated central DA systems in avoidance. For instance, White and colleagues, trained rats to avoid footshock by pressing a lever upon detecting a brief auditory sound (White IM *et al.*, 1991). When rats were able generally to avoid shock on over 80-90% of trials, i.p. administration of both the DA antagonist haloperidol and the antipsychotic clozapine, dose-dependently decreased successful avoidance responses. However, such data do not make clear the extent to which specific DA terminal regions are involved in avoidance responding. More recently, explicit evidence for NAcc mechanisms in such tasks was provided (McCullough *et al.*, 1993). Rats were trained to press a lever both to avoid a shock (each lever press delayed shock presentation for 30 s) and to escape shock (lever-pressing during shock presentation terminated the shock). Performance of this task was accompanied by significant increases in extracellular

levels of DA, DOPAC and HVA, measured by microdialysis, in the NAcc. There was a significant correlation between increased DA and avoidance responses, but not escape responses. Bilateral 6-OHDA-induced DA depletion from the NAcc substantially impaired all aspects of the avoidance task: both avoidance and escape lever responses were significantly decreased, and as a result of this more shocks were presented and duration of shocks were increased (McCullough *et al.*, 1993).

Learning and memory. DA fibres in the mesolimbic system project to the septal nucleus where they are known to have an inhibitory effect on septohippocampal cholinergic neurones (Costa *et al.*, 1983). Manipulations of these DAergic projections have been found to affect performance in learning and memory tasks. Results from these studies are mixed, however, and difficult to interpret: some investigators reported that 6-OHDA lesions of the DA projections to the lateral septum facilitated spontaneous alternation in learning tasks (Galey *et al.*, 1985), while others reported deficits in spontaneous alternation (Simon *et al.*, 1986). Given the clear differences in the connections of subterritories of the NAcc this sort of discrepancy is likely to be attributable to differences in lesion placements.

Summary of dopaminergic function

As a result of these as well as many other studies, the central DA systems have been implicated in different aspects of behavioural activation. In general terms, nigrostriatal DA is involved in the facilitation of stimulus-response connections and is particularly involved in the initiation of motor responses to pertinent stimuli. Mesolimbic DA, on the other hand is concerned with the reinforcement of approach responses to significant goals (termed "incentive motivation"). However, it is the way in which these DAergic inputs interact with cortical and other afferents which determines their specific striatal output patterns. Following this, it is the way in which information from separate striatal outputs interact with each

other and with output circuitry in the pons and medulla which is critical to appropriate neuronal integration for relevant motor pattern selection.

4. Integration of striatal outflow information: a role for the PPTg in the control of behavioural outputs

It has been specifically suggested that neurones in the region of the PPTg-nCh act as an interface between the selection of motor outputs by the basal ganglia and associated limbic structures and the activation of pattern generators in the pons, medulla and spinal cord (Mogenson *et al.*, 1980). Anatomical evidence strongly suggests this: first, there are polysynaptic connections between both the dorsal and ventral portions of the striatum and the PPTg (Tulloch *et al.*, 1978; Swanson *et al.*, 1984; Gerfen, 1985; Rye *et al.*, 1987; Heimer *et al.*, 1991; Moriizumi and Hattori, 1992); and second, the PPTg-nCh sends considerable innervation to the spinal cord (Swanson *et al.*, 1984; Rye *et al.*, 1988; Skinner *et al.*, 1990a). However the precise function of these connections remains uncertain due to ambiguous behavioural evidence.

Nucleus accumbens and the PPTg

The separate outputs of the NAcc, delineated by core and shell, both send information to the PPTg. First, the core projects to the GP (Heimer *et al.*, 1991) and this in turn innervates the PPTg directly (Moriizumi and Hattori, 1992) and via the SNr (Rye *et al.*, 1987; Smith *et al.*, 1990; Von Krosigk *et al.*, 1992). Second, part of the output pathway from the shell of the NAcc projects to the subpallidal region, which includes the substantia innominata and the lateral preoptic area (Heimer *et al.*, 1991). It has in fact been suggested that the largest input to this region arises from the NAcc (Mogenson *et al.*, 1983) and the GABAergic neurones in this pathway are considered to be the major route for locomotor information leaving the NAcc (Mogenson and Nielsen, 1983; Mogenson *et al.*, 1983). There are anterograde (PHA-L) and retrograde (fluorescence) axonal transport studies, as well as electrophysiological recording studies, which indicate that the substantia innominata sends a substantial projection to the PPTg and that

neurones from the same PPTg region project in turn to the spinal cord (Swanson *et al.*, 1984). These anatomical data therefore strongly implicate the PPTg as a major output station for the NAcc and as such suggest that it may play an important role in locomotion and/or the expression of motivationally significant behaviours.

Locomotion

It has been specifically suggested that locomotion generated from the NAcc may be relayed through the PPTg to lower motor systems (Swanson *et al.*, 1984; Brudzynski and Mogenson, 1985) and some authors have obtained a blockade in AMPH-stimulated locomotion following kainate (Brudzynski and Mogenson, 1985) or ibotenate (Bechara and Van der Kooy, 1992c) lesions of the PPTg. The results from each of these studies however might be queried, although for separate reasons. In the first study, (Brudzynski and Mogenson, 1985) there is no histological analysis presented for the kainate lesions, but work from this laboratory (Rugg *et al.*, 1992) demonstrated that kainate infused into the PPTg produced very large, non-selective lesions which damaged many other brainstem sites. In the second study (Bechara and Van der Kooy, 1992c) the locomotor activity induced by an s.c. injection of AMPH was only measured for a 2 min period beginning 2 min post-injection. While this may indicate that drug-induced locomotor activity in lesioned rats was slower to be initiated than in controls, it does not show that locomotion *per se* was affected. Indeed, others have found that lesions of the PPTg did not block locomotor activity: NMDA lesions of the PPTg did not block spontaneous locomotion or that stimulated by s.c. AMPH (Olmstead and Franklin, 1992); ibotenate lesions of the PPTg did not depress either the supersensitive response to s.c. APO following bilateral 6-OHDA injections into the NAcc (Swerdlow and Koob, 1987) or the locomotor effects of AMPH injected directly into the NAcc (JS Dunbar and P Winn, unpublished observations); and quisqualate lesions did not block initial spontaneous exploratory locomotion in a

novel environment or the subsequent spontaneous 10 h of nocturnal locomotion (Dellu *et al.*, 1991).

Despite these contradictory results, the PPTg has been (and often still is) implicated as the major component of the mesencephalic locomotor region (MLR) (for instance Mogenson *et al.*, 1989; Skinner *et al.*, 1990a). The MLR is a functionally well-defined area of brainstem from which it is possible to stimulate coordinated machine-like locomotion on a treadmill in the precollicular-postmammillary transected cat and rat (Shik *et al.*, 1966; Nicolopoulos-Stournaras and Iles, 1984; Coles *et al.*, 1989). The exact anatomical locus of the MLR however has been a controversial issue and despite the contradictory lesion data, a number of anatomical factors pointed to the PPTg-nCh as a brainstem component of the MLR. First, the MLR and the PPTg-nCh were thought to be located in the same general region of the pons; second, both sites were known to have descending projections to the spinal cord (Grillner and Shik, 1973; Castiglioni *et al.*, 1978; Rye *et al.*, 1988); and third, the PPTg was known to be an output station for the NAcc (Mogenson *et al.*, 1983; Swanson *et al.*, 1984), the part of the striatum from which locomotion can be directly stimulated with AMPH (Kelly *et al.*, 1975).

A second reason why the lesion data initially failed to discredit the PPTg as a brainstem generator of locomotion arises from the support given to this idea by acute manipulations, even although these have not themselves been straightforward to interpret. Locomotor activity has been successfully reduced following injections of procaine, a local anaesthetic which slows conduction of neuronal action potentials, into the posterodorsal PPTg region (Brudzynski and Mogenson, 1985). Conversely, electrical stimulation of the PPTg (Milner and Mogenson, 1988; Garcia-Rill *et al.*, 1990), or injecting low doses of excitotoxins such as NMDA (Milner and Mogenson, 1988; Garcia-Rill *et al.*, 1990) or kainate (Milner and

Mogenson, 1988) there, elicits locomotion. Collectively, these data appear to support the conjecture that the PPTg-nCh drives locomotion. However, injecting GABA antagonists such as picrotoxin (Milner and Mogenson, 1988; Mogenson and Wu, 1988) or bicuculline (Milner and Mogenson, 1988) into the PPTg have also elicited locomotion and these data conversely suggest that the PPTg-nCh normally has tonic inhibitory effects on locomotion. Of course these data are not wholly incompatible: it is possible that GABAergic inputs to the PPTg-nCh normally inhibit locomotor activity and this inhibition is attenuated by picrotoxin and bicuculline to "elicit locomotion", while the effects observed following excitotoxins or procaine may occur at excitatory outputs from the PPTg-nCh. It is interesting in this regard that electrical stimulation of the PPTg has been reported to either reduce or stimulate muscle tone, depending on the rate of stimulation (Kelland and Asdourian, 1989; Lai and Siegel, 1990).

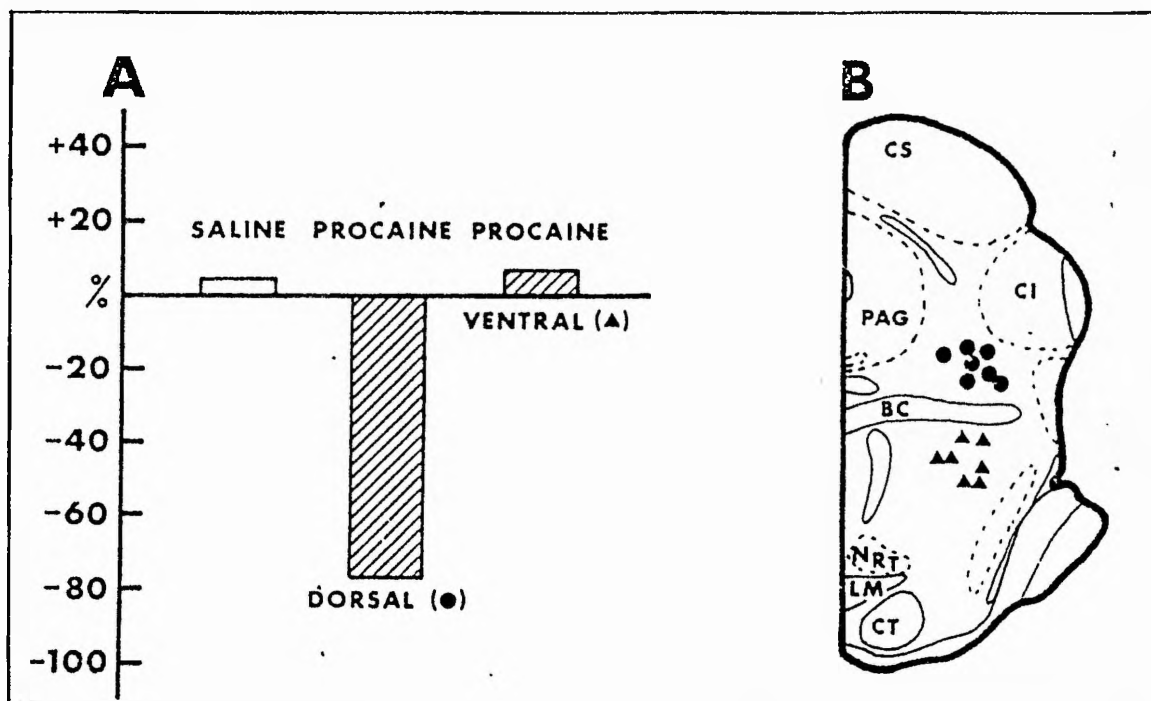
Because of the changes in locomotor activity observed following acute manipulations, the lack of any variation following different excitotoxic lesions was rationalised as resulting from some sort of functional recovery following the lesion. However, there is a compelling alternative explanation. In the acute investigations reported above of the PPTg as a locomotor centre, one injection or electrode was placed at the most posterior part of the nucleus in order to affect that part of the nucleus which is most densely populated with neurones. However, this particular injection site has the unfortunate disadvantage of lying adjacent to the cuneiform nucleus (CnF), a region of the pontine tegmentum which lies dorsal and posterior to the PPTg and which is actually more analogous to the location of the MLR than the PPTg itself (Shik *et al.*, 1966). The study which blocked locomotor activity by injecting procaine (Brudzynski and Mogenson, 1985) demonstrates this problem particularly clearly. The data from these PPTg injections were divided into 2 groups: those which were placed above the superior cerebellar peduncle (scp) at the most posterior part of the PPTg and those which were placed below the scp at

the same anterior-posterior position. Only those injections placed above the scp successfully blocked locomotion elicited from AMPH injections to the NAcc (Figure 4:1), suggesting that it might be neurones in the CnF rather than the PPTg-nCh which were responsible. In the study by Milner and Mogenson (1988), the CnF or the adjacent dorsal and medial portions of posterior PPTg was the location of 26 out of 43 sites which elicited locomotion following electrical stimulation, with the remaining 17 sites placed in various locations in the deep mesencephalic nucleus (DpMe); similarly, following chemical stimulation 16 out of 26 sites which elicited locomotor activity were located in the CnF or immediately adjacent PPTg, 7 were located in the DpMe and 3 in the superior colliculus. Furthermore, electrical stimulation in this study used relatively high currents ($107 \pm 9 \mu\text{A}$) and the locomotor activities observed included jumping, rearing, backward locomotion and elements of defensive behaviour. Such behavioural observations suggest that locomotor components of behavioural stereotypy rather than straightforward "normal" locomotion were being stimulated, and also that adjacent structures to the site of the electrode were co-opted for their production: it is particularly interesting that direct stimulation of the CnF with 50 mM glutamate generates defensive behaviour including freezing, darting and rapid running (Mitchell *et al.*, 1988). The histological figures presented by Garcia-Rill and colleagues (1990) are not sufficiently detailed to dispute the possible role of the CnF rather than the PPTg in producing their observed locomotor effects. Indeed, since the MLR has now been specifically localised to the CnF in the monkey, cat and rat by electrophysiological (Eidelberg *et al.*, 1981; Coles *et al.*, 1989) and anatomical (Moon-Edley and Graybiel, 1983; Shojania *et al.*, 1992) methods as well as the visualisation of *c-fos* induction following treadmill locomotion (Shojania *et al.*, 1992), it would appear that placing the MLR specifically in the PPTg is incorrect.

However, these data do not prove that the PPTg-nCh has no role whatsoever to play in the control of locomotor activity. For instance, it has been suggested that

Figure 4:1

Illustration of the effects of procaine injected into the PPTg, taken from Brudzynski and Mogenson, 1985 (Figure 3). (A) describes the percentage change in AMPH-induced locomotor activity before and after the procaine injection for dorsal versus ventral PPTg; and (B) illustrates the location of the dorsal and ventral injection sites.



gross changes in locomotor activity following PPTg lesions may not occur because a large number of output pathways are likely to be able to modulate locomotion and therefore compensate for any deficit incurred (Swerdlow and Koob, 1987). Broad decisions relating to response selection are likely to have been made long before neuronal information reaches the brainstem, but it is unlikely that one fixed behavioural output will have been chosen at such a high level: it is far more likely that a "response strategy" is in place, from which competing aspects must be selected, probably by the integration of compatible component output channels. The PPTg itself is likely to be one of many pontine nuclei able to collate and integrate information from several output channels. One of these channels may be related to locomotion or muscle tone, while others may contain information related to other particular actions (for example orofacial behaviours, orienting behaviours, components of exploration), the motivational significance of external cues or the incentive to satisfy basic drives. Therefore, while locomotion *per se* may not be affected by PPTg lesions, deficits relating to locomotor activity may be observed when a response requires integration of movement with other components of output information. In this context it is interesting that a connection originating in the NAcc will contain both locomotor and motivational information, which may therefore implicate the PPTg in reward-related outputs and their integration with locomotor behaviour.

Motivation

Lesions of the PPTg with quinolinate or ibotenate have not affected feeding or drinking activities *per se*, which suggests that there are no straightforward changes in established motivational significance of food or water following PPTg lesions (Dunbar *et al.*, 1992). However, just as the absence of locomotor deficits does not rule out a role for the PPTg in certain aspects of locomotor activity, these data do not rule out a role for the PPTg in motivational tasks.

The PPTg is particularly well-suited for co-ordinating competing outputs with each other and with ascending information by means of the considerable integration there between the non-cholinergic and cholinergic neurones. Not only can current outputs be influenced by the most recent information regarding the external environment, but specific details regarding integration of these outputs and execution of particular responses can be relayed back to midbrain, thalamic and cortical sites and modulate their subsequent direction of response strategies. Specifically, the PPTg-nCh could integrate motivational significance with certain situations (for instance, linking environment with reward), pass this information on to PPTg-Ch neurones and they in turn could influence cortical and basal ganglia activity.

Rewarding tasks. PPTg-lesioned rats show deficits in conditioned locomotion and the formation of conditioned place preferences. Conditioned locomotion was assessed in the test environment for 2 min prior to injection of morphine on consecutive test days and increases in locomotor activity normally observed during this period on successive days were blocked by ibotenate infusions to the PPTg (Bechara and Van der Kooy, 1992c). Again, a short observation period was used, but in this case it is probably more appropriate than it was for observing unconditioned locomotion - this method for measuring conditioned locomotion had been used previously (Mucha *et al.*, 1981) and was considered to be a very sensitive procedure. Longer periods of observation would lead to habituation to the test environment and this would compromise the measurement of conditioned locomotion. The increases in conditioned locomotion observed in sham-lesioned rats suggests that they have attributed motivational significance to the test environment as a result of repeated morphine injections there and drive motor output to "approach" this perceived reward. The deficit observed in the PPTg-lesioned group may be due either to a failure to link the environment with reward

or an inability to use the reward-environment information to direct motor output because it has not previously been processed for memory storage.

PPTg-lesioned rats also show deficits in the formation of conditioned place preferences, a phenomenon involving the association of a given stimulus (for instance, drug or food) with an internally-driven incentive and then linking this complex drive with specific environmental cues to direct action. Place preferences to morphine and AMPH were abolished in PPTg ibotenate-lesioned drug-naive rats (Bechara and Van der Kooy, 1989). However, once these rats had acquired a place-preference to morphine, ibotenate infusions did not affect the retention or extinction of this preference, although after lesioning rats were unable to re-acquire the extinguished place-preference to morphine (Bechara and Van der Kooy, 1989). These data therefore support the hypothesis that while the PPTg appears to be directly involved in the acquisition of some component of the stimulus-incentive-environment association, it is not required for its retention or attenuation.

Specific learning disturbances relating to the approach of reward have been observed following quisqualate lesions of the PPTg (Dellu *et al.*, 1991). In a cross maze task where rats had to find the arm baited with food by using visual cues, PPTg-lesioned rats made more errors than controls although this difference was not significant. However in a radial arm maze task, rats with PPTg lesions did make significantly more errors than controls. Although the authors attributed these deficits specifically to cholinergic damage and memory/attention processes, quisqualate lesions in the PPTg are known to damage both cholinergic and non-cholinergic neurones without any particular specificity for the cholinergic portion of the nucleus (Rugg *et al.*, 1992). It is therefore possible that the learning deficits observed following quisqualate lesions resulted not only from disruption of specific cholinergic processes, but also from interactions of the PPTg-nCh output neurones with these processes.

However, the role of the PPTg in the learning of incentive-related tasks may not be so straightforward. Recent data have shown that the requirement of the PPTg for making place preferences is state-dependent: preferences formed by food-deprived or morphine-dependent rats (food- or morphine-paired vs. a neutral compartment; hunger- or withdrawal-paired vs. neutral; food- or morphine-paired vs. hunger- or withdrawal-paired) were unaffected by ibotenate PPTg lesions, while the preferences learned by non food-deprived or morphine-naive rats were blocked by such lesions (Bechara and Van der Kooy, 1992a). Furthermore, by pre-treating morphine-dependent rats with a withdrawal-alleviating dose of morphine 3.5 hr prior to the place-conditioning procedure, PPTg lesions were also able to block formation of place preferences in dependent rats (Bechara and Van der Kooy, 1992b). As a result of these data, Bechara and Van der Kooy (1992a; 1992b) suggested that there could be separate motivational output streams: one (in the absence of deprivation) that depends on a route through the PPTg and another (in the deprived state) that does not.

A possible interpretative problem for these data arises from the fact that the preferences in the deprived and non-deprived rats are likely to have been formed for opposite reasons (Figure 4:2). For naive or non-deprived rats the formation of a preference occurs because of the rewarding or novel significance carried by the drug/food, while for drug-dependent or food-deprived rats it probably occurs because of the significance conveyed by the alleviation of withdrawal or hunger. However, this would suggest that PPTg-mediated incentive mechanisms relate specifically to reward while those relating to the perception of aversive stimuli do not require processing by these neurones.

Aversive tasks. There is reason to believe that the proposed role for the PPTg in processing incentive information *does* extend to avoidance of undesirable stimuli as well as to approach of reward. For instance, in a water maze task, where rats had

Figure 4:2

The 3 different types of pairings used in the conditioned place preference paradigms carried out by Bechara and van der Kooy (1992b). Each type of pairing was carried out 4 times with pairings in different environments taking place on alternate days such that conditioning was carried out for 8 days altogether. For the novel compartment, each rat was injected with either saline (procedure 2) or drug (procedure 3) and returned to the home cage rather than the test environment.

1	Drug-paired compartment	Saline-paired compartment
2	Drug-paired compartment	Novel compartment
3	Novel compartment	Saline-paired compartment

Drug-naïve rats

Implication of different pairings for drug-naïve rats. No supplemental morphine injections were given in the home cage.

1	Drug = REWARD	No drug = NEUTRAL
2	Drug = REWARD	Novel = NEUTRAL
3	Novel = NEUTRAL	No drug = NEUTRAL

Drug-dependent rats

Implication of different pairings for drug-dependent rats. Supplemental maintenance doses of morphine were given at irregular times in the home cage.

1	Drug = NECESSITY	No drug = AVERSIVE
2	Drug = NECESSITY	Novel = NEUTRAL
3	Novel = NEUTRAL	No drug = AVERSIVE

to use spatial cues to escape to a platform from water made opaque by the addition of milk, quisqualate PPTg-lesioned rats could not escape from the water by finding a hidden platform, although when the platform was visible lesioned rats performed as well as controls (Dellu *et al.*, 1991). Similarly, in an active avoidance task, rats with either kainate or ibotenate lesions of the PPTg failed to learn to cross to the opposite compartment during presentation of a warning buzzer/light conditioned stimulus prior to a footshock (Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992). This deficit appeared to be specifically related to the use of incentive information to direct behaviour although the basic motor ability in these rats appeared to be intact: first, they were able to escape normally from the compartment when the shock was presented (Fujimoto *et al.*, 1989); second, rats with ibotenate lesions of the PPTg were also impaired in a passive avoidance task (Fujimoto *et al.*, 1992), where hypoactivity *per se* would be unlikely to produce an avoidance deficit; and third, they were able to perform the task as well as controls if they were given training prior to surgery (Fujimoto *et al.*, 1992). This latter observation also rules out the possibility that changes in visual/auditory reactions following the lesion could account for the results, and there were no apparent differences in perception of pain between lesioned and control rats as threshold levels for vocalization or making a jump response to a footshock did not differ between either kainate- or ibotenate-lesioned rats and controls (Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992). Therefore it would seem that these PPTg-lesioned rats were deficient in their ability to associate the warning buzzer/light conditioned stimulus with its motivational significance in order to implement appropriate avoidance behaviour.

Given the demonstration that PPTg neurones are involved in nicotine-induced nociception (the *response* to painful stimuli, rather than pain itself) (Iwamoto, 1989; Iwamoto, 1991), the absence of apparent differences in perception of pain in PPTg-lesioned rats (Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992) may be surprising. Intra-PPTg injections of nicotine, the non-selective muscarinic agonist

dioxolane or the M₂-selective muscarinic antagonist methoctramine all induced antinociception (Iwamoto, 1989). The effects of intra-PPTg nicotine were attenuated by the nicotinic antagonist mecamylamine, the muscarinic antagonist scopolamine and the M₁-selective muscarinic antagonist pirenzepine, but were potentiated by methoctramine (Iwamoto, 1989). In a later study, it was shown that both methoctramine- and nicotine-induced antinociception could be blocked by pre-treatment of the PPTg with the sodium-dependent high-affinity choline uptake inhibitor, hemicholinium-3 (Iwamoto, 1991). However, ibotenate PPTg lesions did not alter the analgesic properties of morphine (Bechara and Van der Kooy, 1992c) and naloxone did not affect nociceptive effects of intra-PPTg nicotine (Iwamoto, 1991) in tail-flick tests, indicating that endogenous opioid mechanisms in the PPTg are not involved in the mediation of response to pain. Rather, these data suggest that cholinceptive neurones in the PPTg have control over reactions to painful stimuli. Iwamoto (1989) has suggested that intra-PPTg nicotine induces antinociception via a presynaptic mechanism which releases ACh to act at muscarinic M₁ receptors on the post-synaptic membrane. By this route, the antinociception induced by methoctramine might occur by blocking presynaptic M₂ autoreceptors, again releasing ACh to act at the postsynaptic membrane. Nicotine-induced antinociception is also thought to involve mechanisms in the raphe nucleus and pharmacological data suggest that a cholinergic pathway between the PPTg and the raphe, which is tonically inhibited, mediates antinociceptive effects (Iwamoto, 1991). However it appears that although the PPTg receives information relating to painful stimuli - which is necessary in order to recognise predictors of aversion - and moderation of the response to such stimuli can occur through cholinergic mechanisms, pain itself is not mediated there as rats respond to painful stimuli at the same threshold as controls (Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992).

A role for the PPTg in antinociception complements data which implicate the PPTg in the inhibition of the acoustic startle reflex in pre-pulse inhibition. The presentation of a sudden loud auditory stimulus to an animal induces a startle response recognisable by the contraction of facial and skeletal musculature. Pre-pulse inhibition is the term used for the phenomenon of attenuating this startle response by prior presentation of a weak warning stimulus (Hoffman and Ison, 1980). Disruption of pre-pulse inhibition occurs when mesolimbic DA neurones become over-active (Swerdlow *et al.*, 1992) and the PPTg has been implicated in this outflow circuitry via its connections with the ventral pallidum (Heimer *et al.*, 1991). There is direct evidence to support this: first, acoustically-evoked potentials occurring in conjunction with the acoustic startle reflex have been recorded from the PPTg (Ebert and Ostwald, 1991); and second, lesion and electrophysiological data (Leitner *et al.*, 1981; Kelland and Asdourian, 1989) suggest that the PPTg could be directly involved in the muscular inhibition of this reflex following a pre-pulse.

Indeed, integration of the pre-pulse stimulus with the imminent acoustic stimulus can be viewed in a similar fashion to integration of the buzzer/light stimulus with the approaching footshock (Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992) except that the animal cannot escape from the acoustic stimulus and therefore braces itself against the impact. In the same way that PPTg-lesioned rats were unable to use a novel buzzer/light stimulus to predict and avoid footshock, they should also be unable to inhibit the acoustic startle reflex. Preliminary evidence to this effect has been collected (Leitner *et al.*, 1981).

A role for the PPTg in incentive learning. Together, the data which has been reviewed in this section suggest that the PPTg as a whole is required for the utilisation of motivational stimuli in all incentive-driven situations. However, an exception to this rule is in the application of previously learned associations, as

demonstrated in the studies where place preferences and active avoidances learned prior to PPTg lesion were unaffected (Bechara and Van der Kooy, 1989; Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992). This particular piece of information may be the key to understanding why the aversive nature of withdrawal or hunger could still direct formation of place preferences following PPTg lesions, while avoidance of footshock and approach of drug- or food-related reward were impaired. Specifically, the PPTg appears to establish incentive associations between *novel* stimuli such that they can be used immediately to direct outputs via descending cholinergic and non-cholinergic channels and integrated subsequently with ascending cholinergic neuronal signals for modifying forebrain activity. By this mechanism the direction of motor outputs by previously established motivational indicators - such as hunger in food-deprived rats, withdrawal in morphine-dependent rats, or rewarding drug effects in naive rats who learned a preference prior to surgery - will not be affected by lesions of the PPTg. On the other hand, direction of behaviour using novel motivational indicators - such as rewarding drug effects introduced to naive rats after surgery, rewarding drug effects (as opposed to more familiar withdrawal alleviation) in dependent rats, or leaving a compartment in response to a novel buzzer/light stimulus which signals footshock - will be affected by PPTg lesions.

Caudate putamen and the PPTg

Outputs from the CPu contact the PPTg through relays in the GP and SNr (Tulloch *et al.*, 1978; Gerfen, 1985; Rye *et al.*, 1987; Smith *et al.*, 1990; Smith and Bolam, 1991; Moriizumi and Hattori, 1992; Von Krosigk *et al.*, 1992). Although there have been relatively few functional investigations to establish the significance of this connection between the CPu and the PPTg, the anatomical data implicate the PPTg as one of the major output stations for the CPu and suggest that it has an important role to play in the mediation of motor output behaviours related to response selection and initiation. Indeed the most compelling evidence for

functional link between these structures comes from the use of 2-deoxyglucose (2-DG) "metabolic mapping" in primates made parkinsonian with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Mitchell *et al.*, 1989).

The most consistent neuropathological finding in both idiopathic Parkinson's disease (PD) and post-encephalitic parkinsonism is loss of pigmented, DA-containing cells from the SNc (Bernheimer *et al.*, 1973), with associated depletion of the levels of DA in both this structure and the CPu. This loss of DA leads in turn to disordered dorsal striatal activity and it is this which underlies the primary motor manifestations of the disease. Loss of DA from the dorsal striatum in primates following MPTP treatment has been shown, through the accumulation of 2-DG to increase pallidal outflow to the PPTg (Mitchell *et al.*, 1989). The largest change observed in these studies was the greatly increased 2-DG uptake in the terminals of the lateral GP, resulting from increased activity in striatopallidal neurones. Some of the knock-on effects of this increased GABAergic activity were also observed: first in the reduction in GABAergic output from the GP by decreased 2-DG uptake at the terminals in the STn; second, by the increased excitatory output from the STn to the SNr by increased 2-DG uptake in the SNr; and third, by the greatly increased GABAergic output from the SNr to the PPTg by prominent increases in 2-DG uptake in the PPTg. As these outflow pathways from the CPu are thought to be directly related to the rigidity and hypokinesia which form major symptoms in PD, such results implicate inhibition of the PPTg as a major component of parkinsonian symptomatology.

The PPTg in Parkinson's disease and schizophrenia

Several anatomical studies have implicated loss of cells from the PPTg, particularly the PPTg-Ch, in Parkinson's disease (Hirsch *et al.*, 1987; Zweig *et al.*, 1987; Jellinger, 1988; Zweig *et al.*, 1989) and more recently it was suggested that a primary pathological condition in schizophrenia may be an increase in number in

this cell group (Karson *et al.*, 1991). Given the close relationship between cholinergic and non-cholinergic neurones in the PPTg which was emphasised above in the literature concerning outflow from the NAcc, and given also the evidence for disordered activity in the PPTg neurones in Parkinson's disease (Mitchell *et al.*, 1989), such anatomical observations deserve further discussion.

Parkinson's disease

Post-mortem analysis of parkinsonian brains have demonstrated that the PPTg had specific pathological and neurochemical abnormalities in these patients (Hirsch *et al.*, 1987; Zweig *et al.*, 1987; Jellinger, 1988; Zweig *et al.*, 1989). Such reports have all concentrated on the loss of cholinergic neurones. For example, studies of the PPTg-Ch by Zweig and his colleagues have demonstrated an average cell reduction of 60% in 4 cases of PD, and of 40% in 4 cases of Alzheimer's disease (AD)+PD, as compared with age-matched controls, while no significant depletion was seen in cases of AD alone. Lewy bodies were abundant in this area, and neurofibrillary tangles common in all cases of PD and PD+AD but infrequent in controls (Zweig *et al.*, 1987). Similarly, a study by Jellinger (1988) reports a significant decrease in the density of large ($>20\ \mu\text{m}$ [cholinergic]) PPTg neurones by over 50% in PD, with Lewy bodies affecting between 6 and 39% of the remaining cells, while PPTg damage in AD or psychiatric controls was much less severe. Analysis of the PPTg in parkinsonian brains has also been investigated by staining for NADPH diaphorase and assaying ChAT activity there (Hirsch *et al.*, 1987). An estimated 57% of diaphorase-positive neurones in the Ch5 cell group (Mesulam *et al.*, 1983) were lost in PD.

Whether these deficits are directly related to the primary disease process or whether they are secondary to the degeneration of the nigrostriatal pathway is not known. However the demonstration that disordered CPu outputs strongly inhibit transmission in the PPTg (Mitchell *et al.*, 1989) suggests that PPTg-nCh neurones

may switch off activity in PPTg-Ch neurones and eventually lead to the neuronal death observed in post-mortem studies. In any case, abnormalities in PPTg transmission might be partly responsible for some of the symptoms of PD such as cognitive deficits, visuospatial problems and sleep disorders.

Cognitive deficits. Parkinsonian patients have been described as having a syndrome of "bradyphrenia", characterised by diminishing voluntary attention, loss of spontaneous interest, lack of initiative, deficient capacity for mental exertion, subjective and objective fatiguability, and slight memory deficits. Although these cognitive impairments can only occasionally be identified in the early untreated stages of the disease (Lees and Smith, 1983), they are often suggested to be due to loss of caudate DA, which may not actually be sufficient until later stages of the disease process. Given the widespread loss of neurones in the brain, particularly in the later stages of PD, it is likely that other systems, such as brainstem sites, are also involved.

For instance, attentional deficits associated with PD by the delay-independent deficits observed in delayed matching to sample tasks by these patients (Sahakian *et al.*, 1988) are consistent with decreased firing of PPTg-Ch neurones or indeed PPTg-Ch cell loss, either of which could be attributed to disordered PPTg-nCh activity. Given the lack of PPTg-Ch damage in this region in AD patients, it is particularly interesting to note that AD subjects show delay-dependent deficits on such tasks (Sahakian *et al.*, 1988). It is however unlikely that the memory deficits in PD can be attributed to PPTg damage. Tests of recognition and recall memory have shown that parkinsonian patients are impaired on tests of recall but not recognition (Brown and Marsden, 1990). However, the evidence from experimental manipulations in the rat previously outlined suggests that PPTg damage impairs learning related to significant stimuli rather than retrieval of

previously stored information relating to such stimuli (Bechara and Van der Kooy, 1989; Fujimoto *et al.*, 1992).

Visuospatial deficits. Dysfunction of the saccadic, pursuit and vergence systems of ocular motor control are common in PD patients (White *et al.*, 1983b). More specifically, White and colleagues (1983b) have reported (i) prolonged saccadic reaction times and postsaccadic refractory periods; (ii) reduced peak saccadic velocities; and (iii) multiple step, hypometric saccades with abnormally frequent square wave jerks in such patients. These might lead to insufficient or disrupted scanning of visual material and so deficits in performance of visual tasks (Sahakian *et al.*, 1988) might be due to problems in the implementation of scanning procedures rather than to attentional mechanisms *per se*.

Lesions of the SC produce delayed, slowed and hypometric saccades (Schiller *et al.*, 1980) similar to those in PD (White *et al.*, 1983b). Damage to the nigrocolliculoreticular circuit could therefore potentially disrupt saccadic performance and may explain the PD saccadic deficits. The SNr sends major projections to the SC (Redgrave *et al.*, 1992) and Wurtz and Hikosaka (1981) recorded from cells in SNr which modulate with saccades and project to SC. In addition the SNr sends major output to the PPTg-nCh (Rye *et al.*, 1987) while the PPTg-Ch in turn densely innervates the SC (Beninato and Spencer, 1986; Woolf and Butcher, 1986; Hallanger and Wainer, 1988). Over-activity of neurones in the SNr in PD could therefore modulate saccadic eye movements through major inhibition of both the SC and PPTg-nCh, which itself has indirect control over the SC through PPTg-Ch neurones. The role of the LDTg is also interesting to consider in this regard as it has extensive connections with the visual system (see Chapter 1). Whether or not it is also a site of pathology in PD is yet to be determined, but the close anatomical links between the PPTg and LDTg (Semba *et*

al., 1992) could lead to disordered activity in LDTg neurones as a consequence of changes in PPTg-Ch transmission.

Disorders of sleep-wake cycles. An additional characteristic of PD is the disturbance of sleep-wake cycles. The cholinergic neurones of the PPTg and LDTg are implicated in the activation of REM sleep (Steriade and McCarley, 1990) and cholinergic inputs to the thalamus suppress the oscillatory burst firing which is associated with slow-wave sleep (McCormick and Prince, 1986) to produce spike firing associated with being awake. PPTg innervation of the thalamus may also contact the thalamic innervation from the vestibular nuclei which transmits head velocity signals. It is of interest that during all testing protocols relating to vestibular eye movements carried out in darkness, White and colleagues (White *et al.*, 1983a) reported vestibular eye movements punctuated by random periods of wandering movements unrelated to head motion. These wandering eye movements were considered to be similar to the smooth eye movements recorded during the vestibular stimulation of normal sleeping subjects (Melvill Jones and Sugie, 1972) and therefore although these patients all appeared outwardly to maintain alertness, their eye movements suggested incongruously that they were asleep.

Schizophrenia

Recent analysis of post-mortem brain tissue from schizophrenic patients has been carried out using NADPH diaphorase histochemistry (Karson *et al.*, 1991). This study revealed significantly increased numbers of diaphorase-positive cells in the PPTg in schizophrenic brains: from 8500 [SD=1621] in controls to 18275 [SD=8220] in schizophrenics. Although data were reported from only 4 patients, the absence of differences in cell number between patient and control groups in the locus coeruleus suggests that artifacts from histological processing or cell counting were not responsible for the huge increases in numbers observed in the schizophrenic PPTg.

If such differences in cell number are not artificial, it is likely that they occur because of some neurodevelopmental abnormality, such as failure of synaptic pruning of cholinergic neurones akin to that suggested for DA neurones in schizophrenia (Feinberg, 1982). Nielsen and colleagues (Nielsen *et al.*, 1992) have attempted to create an animal model for the development of schizophrenia which replicates the pattern of increased cholinergic cell numbers in the pontine tegmentum. They treated female rats with 5 mg.kg⁻¹ AMPH on days 11-14 of pregnancy and carried out histological analysis on the offspring, comparing the cell numbers in the PPTg and LDTg with those in the offspring of saline treated rats. Diaphorase-positive cell numbers were greatly increased in the LDTg, but not PPTg of the offspring of AMPH-treated rats. Pregnant rats treated with such a dose of AMPH on 4 consecutive days would of course suppress food intake for up to 7 days and as such the results observed may not be a direct consequence of AMPH treatment but instead relate to starvation. However, given the suggestion of high levels of schizophrenia in the offspring of mothers deprived of food during wartime, this type of model may be valid for investigating the development of schizophrenia.

As in Parkinson's disease, interactions between PPTg-Ch and PPTg-nCh neurones may be crucial to understanding the precise role of the PPTg in schizophrenia. Indeed symptoms of the disease such as disruption of pre-pulse inhibition (Braff *et al.*, 1978), postural aberrations (King, 1974), disruption of REM sleep (Tandon and Greden, 1989) and production of REM activity during waking to produce hallucinations (Vanni-Mercier *et al.*, 1989) could all result at least partly from the disruption caused by too many PPTg-Ch neurones on striatal DA activity, disordered striatal outputs running through the PPTg-nCh and subsequent interactions of each of these.

5. General Methods

Animals

All rats were male Lister hooded and unless otherwise stated were bred in-house. They were maintained under controlled conditions with a 12:12 hr light/dark cycle and were individually housed. All rats were allowed *ad lib* access to tap water and to food (SDS maintenance diet no.1 chow pellets), except where food intake was specifically restricted in conditioned reinforcement experiments. In these experiments rats were fed each day at least 1 hr after completion of training. Body weights were monitored and rats were maintained at approximately 85% of their free-feeding weight.

Anaesthesia

Three different anaesthetics were used for the following experiments. Each is associated with its own particular difficulties and was chosen in relation to the surgery to be carried out; specific examples are clarified below.

Avertin

Rats are injected i.p. with 10 ml.kg⁻¹ Avertin (10 g tribromoethanol/5 g tertiary-amyl-alcohol; 10 ml of this concentrate then dissolved in 40 ml ethanol and 450 ml 0.9% saline/0.01M PO₄ Buffer; pH 7.2 - 7.4). On removal from the stereotaxic frame, rats were immediately injected i.p. with 5ml 6% glucose / saline in order to minimize the gastrointestinal irritant effects of the anaesthetic.

Avertin is a fast-acting anaesthetic which allows typically 45 min surgical anaesthesia. If surgery must be continued for slightly longer periods there are no particular problems associated with giving a second "top-up" injection. Avertin is suitable for use when making excitotoxic lesions as it is not thought alter the actions of excitotoxins by interacting with glutamate channels. The relatively quick

recovery following Avertin also makes it suitable for use when implanting guide cannulae. However, the major drawback with the use of Avertin is its gastrointestinal irritant properties: rats anaesthetised with Avertin are likely to develop a gastrointestinal syndrome ("bloat") post-operatively which is characterized by loss of weight, cessation of feeding and defecation and a swollen appearance in the gut region. Development of bloat is always fatal, although its incidence can be minimized by filtering the anaesthetic before use and by giving each rat 5 ml 6% glucose/saline immediately on completion of surgery as described above. Such an injection dilutes the irritant effects of any anaesthetic residue remaining in the i.p. cavity and ensures that the movement of ionic currents across gut membranes are restored.

Xylazine / ketamine

Rats are injected i.p. with 7 mg·kg⁻¹ xylazine (0.35ml·kg⁻¹) and ketamine (1 ml·kg⁻¹). Ketamine hydrochloride ("Vetalar", 100 mg·ml⁻¹, Rogar/STB) is a rapid onset, non-barbiturate. For surgical procedures requiring muscle relaxation, it is recommended that Vetalar is used in conjunction with xylazine ("Rompun", 20 mg·ml⁻¹, Haver) which is a sedative with muscle-relaxant properties. The combination of these drugs typically gives 30-40 min surgical anaesthesia and full recovery occurs approximately 40 min later. The use of ketamine is avoided when making excitotoxic lesions as it is known to interact with NMDA receptor actions (Anis *et al.*, 1983). The use of xylazine when making 6-OHDA lesions following pargyline pre-treatment should also be avoided: both xylazine and pargyline have mild hypertension-inducing effects, but the interaction of these treatments has additive effects on hypertension and can be fatal (Weißenborn, 1992). There may also be problems associated with the use of this combination anaesthetic for surgery involving rats on food-intake restrictions where modification of the dose is necessary.

Sodium pentobarbitone

Rats are injected i.p. with 60 mg·kg⁻¹ sodium pentobarbitone ("Sagatal", 60 mg·ml⁻¹, RMB Animal Health Ltd.). Sodium pentobarbitone is a barbiturate anaesthetic and is typically slower onset than the others described above, allows approximately 60-90 min surgical anaesthesia with full recovery taking up to 3 hr. The active dose window is fairly narrow, is temperature dependent and subsequent top-ups can lead to respiratory difficulty. Sodium pentobarbitone is known to block kainate and quisqualate channels in the cortex (Marszalec and Narahashi, 1992) and also attenuates the toxicity of quinolinate but not ibotenate in the pons (Inglis *et al.*, 1993). The use of barbiturates for making excitotoxic lesions is therefore not advised. However, at doses of 50-55 mg·kg⁻¹ i.p., barbiturates are suitable for implanting guide cannulae in rats with restricted food intake.

Surgical procedures

Excitotoxic lesions

Ibotenate (Cambridge Research Biochemicals) and quinolinate were prepared as 0.12 M solutions in sterile phosphate buffer (pH 7.4); the final pH of the solutions were adjusted with 2M NaOH to approximately pH 7.3 / pH 7.4. Injections of excitotoxins were made using a 1 µl SGE syringe mounted on a stereotaxic frame, with the needle bevel pointing forwards. All excitotoxic lesions were made with the skull level.

For the PPTg, 2 injections were made in each hemisphere at the following stereotaxic coordinates: 0.8 mm anterior to the interaural line, \pm 1.6 mm from midline and 7.0 mm below skull surface (posterior PPTg); and 1.5 mm anterior to the interaural line, \pm 1.7 mm from midline and 7.8 mm below skull surface (anterior PPTg). Half the rats had anterior then posterior PPTg infusions, half posterior then anterior. Ibotenate, quinolinate or phosphate buffer was delivered in a volume of 0.2 µl (24 nmol) to each site in 0.02 µl steps at 10 sec intervals (100 sec). The

needle was then left *in situ* for 200 sec to allow diffusion of solution from the needle tip.

For the DpMe there was one placement in each hemisphere. The coordinates were 2.4 mm anterior to the interaural line, ± 1.7 mm from midline and 6.6 mm below skull surface. For the infusions, 0.4 μ l (48 nmol) was delivered in 0.02 μ l steps at 10 sec intervals (200 sec), and the needle left *in situ* for 300 sec.

For bilateral lesions, rats had 2 separate unilateral operations. These were usually 24 h apart, although when trained food-deprived rats were operated, this was increased to 48 h to maximize survival. Previous experience has shown that ibotenate infused bilaterally into these regions in one surgical procedure is fatal. Rats were given 2-3 weeks to recover from surgery before behavioural testing began.

Implantation of guide cannulae

During the surgical procedure, guide cannulae were secured in place by dental cement and skull screws, and were occluded by stylets to preclude obstruction. Guide cannulae were constructed from 23 ga stainless steel injection needles (Microlance: Surgical supply services, Cumbernauld). They were severed from the base of the needle such that the guide was exactly 11.5 mm in length and had a sharp, pointed end. The portion of the guide near the other, straight end, was roughened slightly so that it would give better purchase to the dental cement during implantation. Stylets were made from hard 30 ga stainless steel wire (Cooper's Needleworks, Birmingham). Lengths of wire were cut with wire clippers and bent in half at an angle of 90°. One end was cut to fit snugly within the length of the guide and the other was cut to a length of 0.3/0.4 mm. This protruding end not only prevented the stylet from being inserted too far, but also acted as a lever

for its removal. Following construction guide cannulae and stylets were cleaned in a sonicator and stored in absolute alcohol until required.

Substantia nigra / Ventral tegmental area. Rats were placed in a stereotaxic frame with the incisor bar 5.0 mm above the interaural line (De Groot) and implanted with stainless steel guide cannulae terminating 2.0 mm above the desired site. The co-ordinates (Pellegrino *et al.*, 1979) for SN, guide cannula placements were A-P: 2.8 mm posterior to bregma; Lateral: ± 2.0 mm from the midline; Vertical: -5.5 mm from dura (injection site -7.5 mm from dura). For VTA guide cannula placements, co-ordinates were A-P: 2.6 mm; Lateral: ± 1.0 mm; Vertical: -5.5 mm from dura (injection site -7.5 mm from dura).

Nucleus accumbens. Rats were placed in a stereotaxic frame with their skulls level (incisor bar approximately 3.3 mm below the interaural line) and implanted with stainless steel guide cannulae terminating 1.0 mm above the nucleus accumbens. The guide cannula co-ordinates (Paxinos and Watson, 1986) were A-P: 2.0 mm anterior to bregma; Lateral: ± 1.5 mm from the midline; Vertical: -5.5 mm from dura (injection site -6.5 mm from dura).

Intracranial microinjection

Microinjection needles were made from 30 ga stainless steel hollow wire (Cooper's Needleworks, Birmingham). 25 mm lengths of this wire were cut, taking care not to crimp the ends, and a 0.5 m piece of PP10 polyethylene tubing (Portex tubing: Surgical supply services, Cumbernauld) was glued to one end of each using an epoxy resin adhesive ("Araldite", CIBA-Geigy). Once this had hardened securely, each needle was measured and a piece of PP10 polyethylene tubing was added to each so that they were either exactly 13.5 mm (SN/VTA) or 12.5 mm (NAcc) in order to reach their required sites.

Injection needles were connected via the polyethylene tubing to an SGE 10 μ l syringe mounted in an infusion pump. All injection solutions were back-filled. Each dose of drug (calculated in terms of the salt) and the saline vehicle control were administered in a random counterbalanced order and successive injections were separated by a minimum of 48 hr. All drugs were supplied by Sigma. Rats were lightly hand-held while microinjections were carried out: stylets were removed and injection needles lowered gently into each guide cannula. The injection volume for SN and VTA was 0.5 μ l infused over 60 sec and the cannula was then left in position for a further 30 sec to allow for drug diffusion away from the tip; for NAcc the injection volume was 2 μ l infused over 240 sec with a further 120 sec *in situ*. Following this, injection needles were removed and clean stylets inserted into the guides prior to placing the rat in the test apparatus. On habituation or training sessions immediately prior to testing, sham-microinjections were carried out to accustom rats to the procedure. These were identical to the genuine injection procedure, except that instead of placing injection needles into the guide, a stylet was placed there for the duration of the "injection".

Behavioural testing procedures

Unconditioned feeding

Following recovery from surgery rats were habituated to the test cages and procedure. The test cages were housed away from the home cages in a separate light- and temperature-controlled room. Rats were placed in individual cages with sawdust-covered floors for 60 min prior to and 60 min following microinjection.

Behavioural observations

In some studies rats were observed by the method of Fray and colleagues (Fray *et al.*, 1980). The presence of the following responses was recorded once every five minutes in a 10 sec observational period:

- (a) still/asleep - lying or sitting; not engaged in any activity.
- (b) locomotion - all 4 legs moving for >3 sec.
- (c) rearing - both forepaws raised from the cage floor.
- (d) sniffing for >3 sec.
- (e) grooming (forepaw) - forepaw grooming of the face and head for >3 sec.
- (f) grooming (other) - grooming other than that in (e) above for >3 sec.
- (g) gnawing polystyrene chips for >3 sec.

Conditioned reinforcement

The apparatus consisted of four double-lever operant chambers (0.25 m x 0.23 m x 0.20 m, Campden Instruments), each housed in a soundproof outer chamber with a ventilation fan to minimize external noise. A pellet dispenser delivered food pellets (45 mg dustless precision food pellets, Campden Instruments Ltd.) into a food hopper situated between two levers - each 38 mm wide, 20 mm from the side and 50 mm above the grid floor - and access to the hopper was via a hinged plexiglas panel, 55 mm high by 70 mm wide. The hopper tray could be illuminated by a light located behind the panel above the food tray and the operant chamber could be illuminated by a light on the ceiling (both lights 2.5 W, 24 V). A Eurobeeb microcomputer system ("Beetle", Paul Fray Ltd, Cambridge) controlled the activities of the operant chambers and was responsible for data collection.

Rats were food-deprived to 85% of their baseline feeding weight prior to commencing training. The animals were introduced to the test apparatus in two 10 minute pre-training sessions. In each of these, 12 food pellets were placed in the hopper with the hopper light on and house lights off. In the first session the plexiglass panel was taped back, but during the second session all rats pushed the panel to gain access to the food pellets.

Training phase. During training, rats were subjected to a classical conditioning paradigm in which they learned to associate a compound stimulus (house light off / hopper light on / click of food dispenser) with delivery of a food pellet. Initially the delay between presentations of the compound stimulus was 10-15 sec and rats were trained not to respond prematurely by ensuring that no pellets were delivered for at least 3 sec if an early panel push was recorded. Training progressed on an increasing random-time schedule until a delay of 28-32 sec was achieved between presentations of the compound stimulus. Rats achieved a criterion of panel pushing "correctly" (i.e. in response to the compound stimulus) on greater than 80% of their panel pushes for at least 5 consecutive sessions before and after surgery. The computer recorded the number of premature panel presses as well as the number of right and left lever presses, although lever-pressing at this stage did not initiate any programmed features.

Testing phase. For the test phase of the experiments, the food pellets were removed from the hopper. Responding on the conditioned reinforcing (CR) lever resulted in the presentation of the compound stimulus without food, while responses on the non-conditioned reinforcing (NCR) lever had no programmed consequences. The lever designated to produce the CR was counterbalanced over subjects in each group but remained the same for each rat throughout testing. In a preliminary "autoshaping" session, rats were placed in the chambers and remained there until ten correct responses on the CR lever were recorded. Test sessions began the following day: following microinjection, rats were placed in the operant chambers for a 30 min test session. Total responses on each lever, total panel pushes and frequency of CRs were recorded at 3 min intervals during the test sessions.

Histological Analysis

Verification of microinjection sites

On completion of the behavioural testing, rats were killed by an i.p. injection of 1.5 ml "Euthatal" (sodium pentobarbitone, 200 mg·ml⁻¹) and perfused transcardially with saline followed by 4% formalin. The brains were stored in 4% formalin before serial sections of 50 µm were cut on a bench microtome and stained with cresyl violet according to normal histological procedures. This permitted an examination of cannula tracks and verification of microinjection sites using a Leitz "Diaplan" microscope.

Analysis of PPTg and DpMe excitotoxic lesions

Rats were treated i.p. with 0.5 ml heparin (5000 USP units·ml⁻¹ sterile saline) 30 min prior to an i.p. injection of 1.5 ml "Euthatal" (sodium pentobarbitone, 200 mg·ml⁻¹). They were then perfused transcardially at a rate of 20 ml·min⁻¹ with phosphate buffered saline (PBS) at 37°C, followed by at least 300 ml fixative (4% paraformaldehyde in 0.1 M phosphate buffer). The brains were removed and post-fixed in the same fixative for 60 min at room temperature, before being cut into 50 µm sections on a freezing microtome: coronal sections were taken from the anterior portion of the cerebellum through to the posterior thalamic nuclei. Sections for Nissl substance (cresyl violet) were stained as above.

Enzyme histochemistry. Sections were stained for nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) using a modification of the method of Vincent and colleagues (Vincent and Kimura, 1992). Following sorting, sections were washed with 30% sucrose for 60 min followed by 3 x 5 min in 0.1 M phosphate buffer (PB). Sections were transferred to a 24-well tissue culture plate where they were incubated at 37°C for 30 min - 1 h in a solution containing 0.1 mg·ml⁻¹ nitroblue tetrazolium (Sigma) and 1 mg·ml⁻¹ βNADPH (tetrasodium salt type 1, Sigma) in 0.3% Triton X-100 / 0.1 M PB. The incubation

volume was approximately 0.5 ml in each well. Finally, sections were rinsed in 0.1 M PB and mounted on chrome alum gelatin-subbed slides and air dried overnight. As the stain is not stable in xylene, coverslips were applied with Gelatin.

Immunohistochemistry. Analysis of choline acetyltransferase (ChAT) -positive and tyrosine hydroxylase (TOH) -positive neurones was carried using previously established procedures (Dunbar *et al.*, 1992; Rugg *et al.*, 1992). Following sorting, sections were washed with 30% sucrose for 60 min and then washed 5 x 5 min in PBS at room temperature. Incubations with antibodies and other reagents were carried out in 24-well tissue culture plates in a volume of 0.3 - 0.5 ml. Up to 6 sections were incubated per well. For washing sections were transferred to a container with a fine net bottom. All incubations were carried out at room temperature on a flat-bed shaker, with the exception of initial incubations with the primary antibodies for ChAT and TOH, which were at 4 °C. Sections were placed in a blocking solution (20% normal goat serum, 0.1% Triton X-100 in PBS) for 60 min and then washed 3 x 5 min with PBS. They were incubated with anti-ChAT (from rat-mouse hybridomas, Boehringer) 1:100 in antibody diluting solution (ADS) or anti-TOH (from mouse-mouse hybridomas, Boehringer) 1:50 in ADS for ~15 hr followed by 5 x 5 min washes with PBS. The ADS used was 0.1% normal goat serum and 0.1% Triton X-100 in PBS. The sections were then incubated with anti rat IgG (1:50 in ADS) (sheep, Sera-lab) for ChAT and anti mouse IgG (1:30 in ADS) (sheep, Sera-lab) for TOH for 1 hr and washed 5 x 5 min in PBS. Following the wash sections were incubated with monoclonal rat peroxidase anti-peroxidase (1:60 (Sera-lab) or 1:1000 (ICN) in ADS) for ChAT and monoclonal mouse peroxidase anti-peroxidase (1:100 (Sigma) in ADS) for TOH for 60 min and washed 5 x 5 min in PBS. The IgG and peroxidase anti-peroxidase incubations were repeated in the same order for the same length of time with PBS washes between each step. Finally, sections were incubated with 0.05% diaminobenzidine for 15 min (1 ml/well) followed by the addition of 10 µl/well 1% H₂O₂ and a

further incubation of 5-10 min. The sections were washed 5 x 5 min in PBS, mounted on glass slides and air dried. Coverslips were applied with DPX.

Lesion Assessment. All sections were inspected using a Leitz Diaplan microscope fitted with a Sony DXC-3000P video camera for visualization of sections on a high resolution colour monitor. Lesions were identified in cresyl violet stained sections by the presence of gliosis and degenerating neuronal somata. For each rat an estimate of neuronal loss in structures around the lesion site was made on a scale of 0 to 4 in both cresyl violet and TOH sections, where 0 indicated no loss of neurons, 1 that 0-30% of neurones were destroyed, 2 that 30-60% of neurones had been lost and 3 that 60-90% of neurones in that structure were destroyed, and 4 that over 90% of neurones were damaged. Neuronal loss in the PPTg was directly evaluated by cell counts in this region for both ChAT and diaphorase.

Statistical Analysis

Where appropriate, data were analysed parametrically using analysis of variance (ANOVA) or Student's t-test. Time-based data were transformed to achieve homogeneity of variance (Winer, 1971). *Post hoc* analysis was carried out where necessary using Tukey's method for multiple comparisons.

6. Control of striatal dopamine by cholinergic neurones in the pons: effects of cholinergic stimulation of substantia nigra on unconditioned behaviour

Introduction

Microinjection of cholinergic substances into the rat anterior SN produces dose-dependent increases in activities for which the animal has both a low baseline rate and a positive predisposition (Winn and Redgrave, 1979; Winn and Redgrave 1981; Winn *et al.*, 1983; Winn 1991). Most of these previous studies used carbachol and it has been demonstrated clearly that those doses of carbachol which affect food intake (0.1 and 0.5 $\mu\text{g}/0.5 \mu\text{l}$) have no effect on other unconditioned activities such as gnawing, locomotion, grooming, sniffing or rearing (Winn *et al.*, 1983; Winn 1991).

Until relatively recently carbachol was considered to be a purely muscarinic agonist and therefore behaviours initiated by cholinergic stimulation of the SN were thought to occur via muscarinic receptor activation. This point of view was complemented by evidence that application of ACh/eserine mixtures to SN can be blocked by atropine (Winn *et al.*, 1983). However, nicotinic receptors are also present on the postsynaptic terminals in SNc (Clarke and Pert, 1985; Clarke *et al.*, 1985) and carbachol does have nicotinic actions, although these should be kept firmly in context. First, from systemic studies it is likely that the nicotinic actions of carbachol are very minor compared to its muscarinic actions in the dose ranges typically used (Bowman and Rand, 1980, p. 10.10); second, natural alkaloids such as muscarine or pilocarpine, which have dominant muscarinic actions, were previously avoided due to their extremely poisonous nature; and third, there is currently no substitute for carbachol in terms of a muscarinic agonist which is selective for M_1 receptors (Watson and Girdlestone, 1993), the subtype known to be present in SN (Cortés and Palacios, 1986; Schwartz, 1986). The nicotinic

mechanisms in SN and their impact on behaviour can be investigated directly by administration of nicotine in unconditioned feeding paradigms similar to those used for carbachol. Indeed activation of behaviour by intranigral nicotine might have a qualitatively different pattern to that following carbachol administration there: it has been suggested that ACh acts as a 'fast' neurotransmitter through the nicotinic receptor and as a 'slow' neurotransmitter through the muscarinic receptor (Kemp *et al.*, 1977). Nicotinic stimulation is excitatory in nature whereas muscarinic stimulation can be excitatory, inhibitory or have potentiating effects on the neuronal membrane with respect to further binding by other molecules. It is therefore important to ascertain whether nicotinic and muscarinic activation of SN stimulate different types of behavioural responses.

Given the existence of both muscarinic and nicotinic receptors on SNc DA neurones, it is also important to explore the behavioural effects of normal interactions between them when ACh is released into the synapse. These interactions can begin to be examined through the use of AChE inhibitors, which act by blocking breakdown of ACh to increase levels of endogeneous ACh in the synaptic cleft. Application of the AChE inhibitor eserine (also known as physostigmine) to the SN has previously been shown to influence behavioural output, presumably through direct actions of ACh. At very high doses compulsive gnawing has been observed (Smelik and Ernst, 1966) while at lower doses feeding can be stimulated (Winn and Redgrave, 1981) although this latter result has proved difficult to replicate (J. Hagan, unpublished observations).

Therefore, this study fully re-examined the effects of intranigral eserine on unconditioned behaviours, and compared the effects of eserine with those of carbachol and nicotine. Given that previous data collected following eserine injections have been inconsistent, the stimulatory effects of a second reversible AChE inhibitor, neostigmine, were also examined. For this, two separate dose

ranges were tested as the strength of action of neostigmine in SN had not previously been explored. Feeding of dry macaroni, feeding of normal lab chow and drinking of tap water were measured by various different parameters (for instance amount consumed, latency to begin and time spent consuming). A detailed examination of other unconditioned activities was carried out simultaneously using the method of Fray and colleagues (Fray *et al.*, 1980).

This experiment was carried out in conjunction with Dr. Graham Parker and was also reported in his PhD thesis. This is an independent report of the same data-set. The surgical, microinjection and histological procedures were carried out by Parker, while I recorded food and water intake, scored the parameters of feeding and classified other behaviour on the Fray checklist. We were both involved in the statistical analysis. A partial account of these data was published recently in *Psychopharmacology*, Volume 112, pp. 242-248: GC Parker, WL Inglis and P Winn, "A comparison of behaviour following stimulation of the anterior substantia nigra by direct and indirect cholinergic agonists".

Methods

Animals

55 male Lister hooded rats with body weight at the time of surgery $259.7 (\pm 15.28$ [SD]) g.

Surgery

Rats were anaesthetised with Avertin and implanted unilaterally with a stainless steel guide cannula positioned above the substantia nigra.

Intracranial microinjection

Rats were arbitrarily assigned to one of five drug groups. Each group was given 4 injections to the SN of saline vehicle and either carbamylcholine chloride

(carbachol) (0.1, 0.5, 5.0 μ g), nicotine hydrogen tartrate (0.1, 0.5, 5.0 μ g), eserine hemisulphate (2.5, 5.0, 10.0 μ g), neostigmine methyl sulphate (0.1, 0.5, 1.0 μ g) or neostigmine methyl sulphate (1.25, 2.5, 5.0 μ g).

Behavioural testing procedure

Rats were observed in the unconditioned feeding paradigm (see General Methods). During the habituation and test sessions rats had free access to weighed amounts of tap water, normal lab chow, dry macaroni (1470 Kj/100 g, 13.0 g protein/100 g) and polystyrene packing chips. They were transferred to freshly provisioned cages every 30 min such that during the test period feeding and drinking could be monitored regularly before and after microinjection. In addition to recording food and water intake, the following parameters of feeding were scored:

- (a) latency to feed (min) - the time taken to initiate the first bout of feeding. A bout was defined as any period of sustained eating lasting 30 sec or more.
- (b) duration of feeding (min) - total time spent eating.
- (c) number of bouts - the frequency of bouts initiated in the test period. Termination of a bout was marked by a minimum of 30 sec without feeding.
- (d) bout length (min) - mean bout length for each rat.
- (e) rate of eating (g/min) - the amount eaten divided by the duration of feeding.

An attempt was also made to quantify each rat's general behaviour by the previously described method of Fray and colleagues (Fray *et al.*, 1980). This method has previously been used to evaluate behaviour following carbachol microinjection to the SN (Winn *et al.*, 1983). Other responses were also noted, including seizure-like activity.

Statistical Analysis

Food and water intake data were analysed parametrically by ANOVA where dose of drug and time period were the dependent variables. For time-based (latency or

duration of responding) data, the raw scores were logarithmically transformed to preserve homogeneity of variance (Winer, 1971). The remaining behavioural data, although collected by the observational method of Fray (Fray *et al.*, 1980), could not be analysed by the Information Statistic which those investigators used. The information statistic requires that all measures be independent, but the data in the present study involved repeated measures, each rat serving as its own control. Therefore the control mean count and standard deviation for each behavioural category were calculated from the saline data from all 5 drug groups for the first 30 min and the first 60 min post-injection. At each experimental dose the count for a behavioural category for individual rats was deemed to be significantly different to control if greater than the mean control score for that behaviour plus 2 standard deviations.

Results

Histological analysis

Figure 6:1 presents representative sections indicating injection placements. Following histological examination, 46 rats were found to have injection sites in or immediately adjacent to SN. The close association between SNc and SNr formed by the descending dendrites of SNc DA neurones means that an injection site in SNr will affect SNc neurones. Those rats found by histological inspection to have misplaced cannulae - that is outside the SNc and SNr - were discarded from the behavioural analyses and are shown on Fig. 6:1 as misplaced cannulae. Although no formal examination was made, inspection of Fig. 6:1 suggests that there were no differences between effective cannulae placements in any of the different drug groups.

Eating and drinking in response to cholinergic stimulation

Table 6:1 shows the breakdown of mean intake of dry macaroni following microinjection. Analysis of macaroni consumed post-injection revealed significant

Table 6:1

The mean intake of dry macaroni during each 30 min and during 60 min following unilateral microinjection of various doses ($\mu\text{g}/0.5 \mu\text{l}$) of cholinergic substances and saline control into rat substantia nigra. Significant effects (marked [*]) of dose ($p < 0.05$ compared to saline) with no effect of time period were found in the carbachol, nicotine and neostigmine (high) groups and there was an effect of dose only over 0-30 min in the neostigmine (low) group. There were no dose effects in the eserine group.

	Dose	0-30 min	30-60 min	0-60min
Carbachol	0.0	1.78 ± 0.30	1.12 ± 0.24	2.90 ± 0.44
	0.1	2.26 ± 0.17	1.48 ± 0.30	3.74 ± 0.43
	0.5	2.51 ± 0.34 *	2.05 ± 0.33 *	4.56 ± 0.44
	5.0	1.15 ± 0.39	1.96 ± 0.41	3.11 ± 0.56
Nicotine	0.0	1.86 ± 0.25	1.36 ± 0.28	3.22 ± 0.45
	0.1	2.14 ± 0.11	2.03 ± 0.22	4.17 ± 0.24
	0.5	2.52 ± 0.35 *	2.00 ± 0.28 *	4.52 ± 0.39
	5.0	1.85 ± 0.24	1.70 ± 0.33	3.55 ± 0.30
Eserine	0.0	1.63 ± 0.28	1.79 ± 0.26	3.42 ± 0.33
	2.5	1.80 ± 0.25	1.70 ± 0.41	3.50 ± 0.39
	5.0	1.64 ± 0.32	2.04 ± 0.36	3.68 ± 0.52
	10.0	1.30 ± 0.35	1.96 ± 0.27	3.26 ± 0.49
Neostigmine (low)	0.0	1.83 ± 0.30	1.47 ± 0.39	3.30 ± 0.30
	0.1	2.82 ± 1.15 *	1.12 ± 0.46	3.94 ± 0.27
	0.5	1.88 ± 0.45	1.30 ± 0.39	3.18 ± 0.65
	1.0	1.43 ± 0.42	1.40 ± 0.28	2.83 ± 0.48
Neostigmine (high)	0.0	2.25 ± 0.24	1.29 ± 0.29	3.54 ± 0.49
	1.25	1.20 ± 0.45	1.35 ± 0.30	2.55 ± 0.70
	2.5	0.72 ± 0.38	0.92 ± 0.27	1.64 ± 0.55
	5.0	0.22 ± 0.16 *	0.46 ± 0.18 *	0.68 ± 0.33

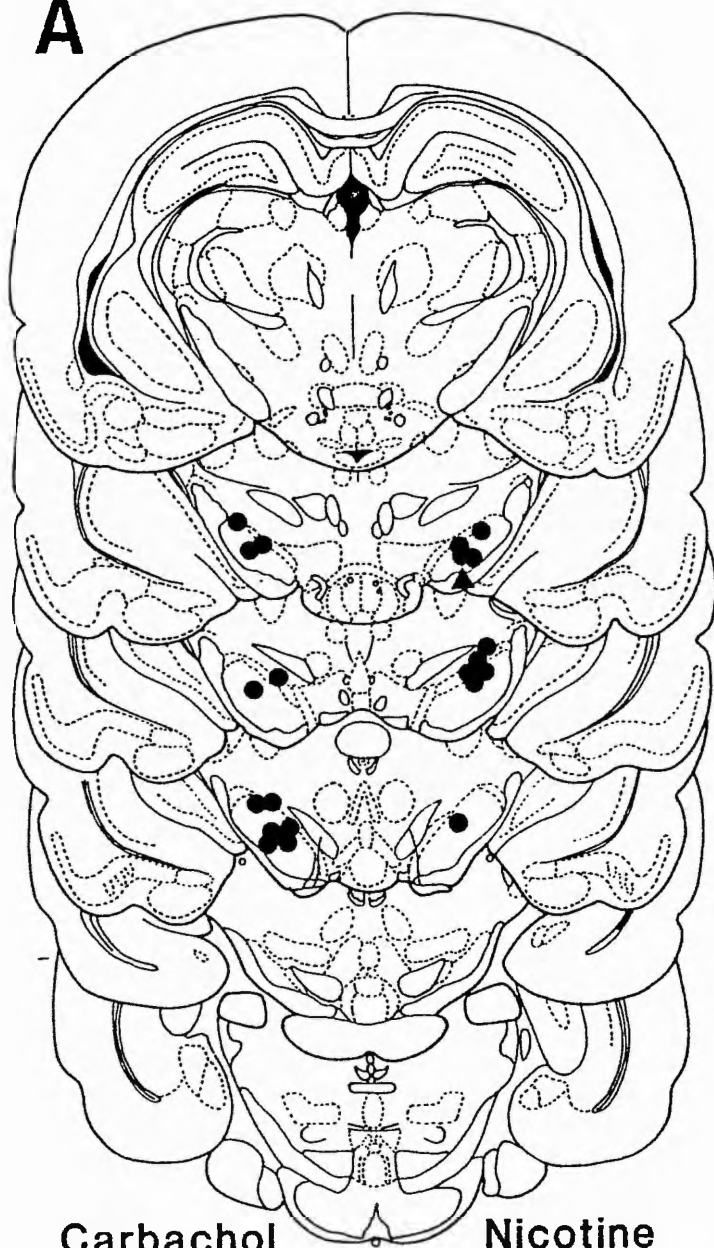
Figure 6:1

Representative sections, redrawn from the atlas of Paxinos and Watson (1982) showing nigral (filled circles) and misplaced (filled triangles) cannulae. The placements of cannulae delivering the different drugs are shown separately, collapsed onto right or left hemispheres. The neostigmine placements show both high and low doses. There were no clear differences between the sites of injection of the different drugs.

Figure 6:2

Mean amounts of food (dry macaroni) consumed in 0-30 min and 30-60 min following unilateral microinjection of cholinergic drugs into the rat substantia nigra. Graphs which illustrate significant effects are marked (*) (see text for details).

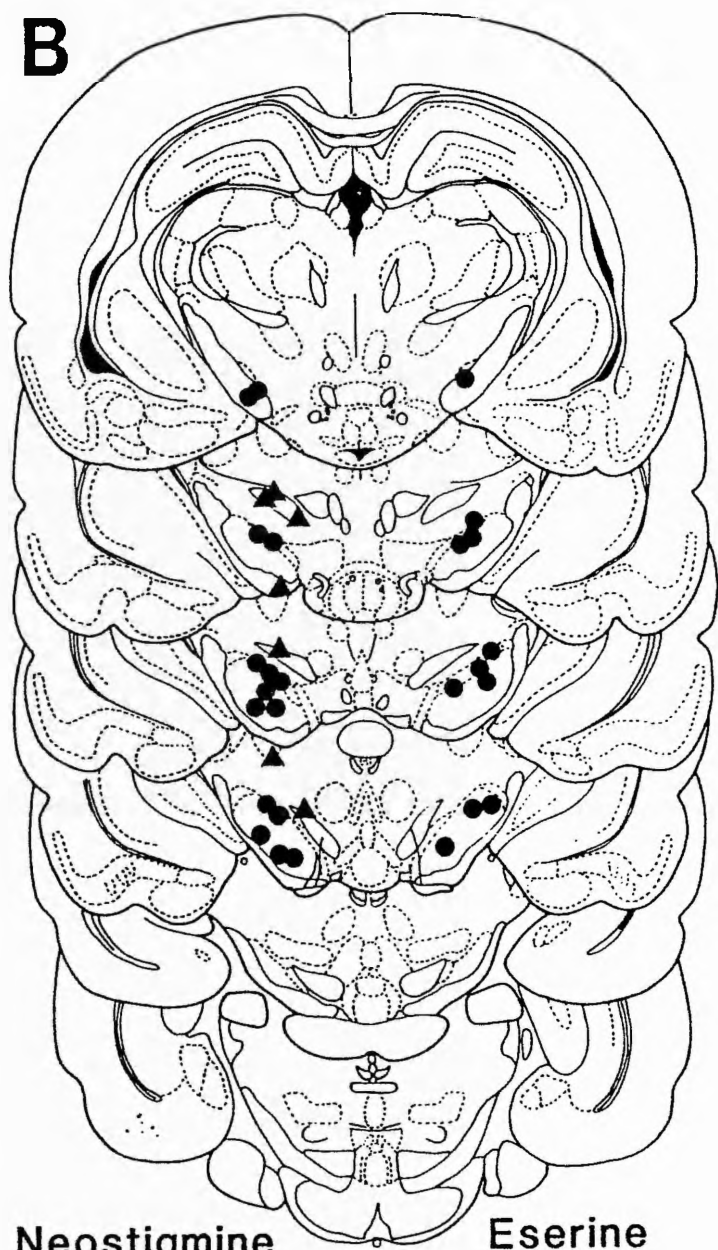
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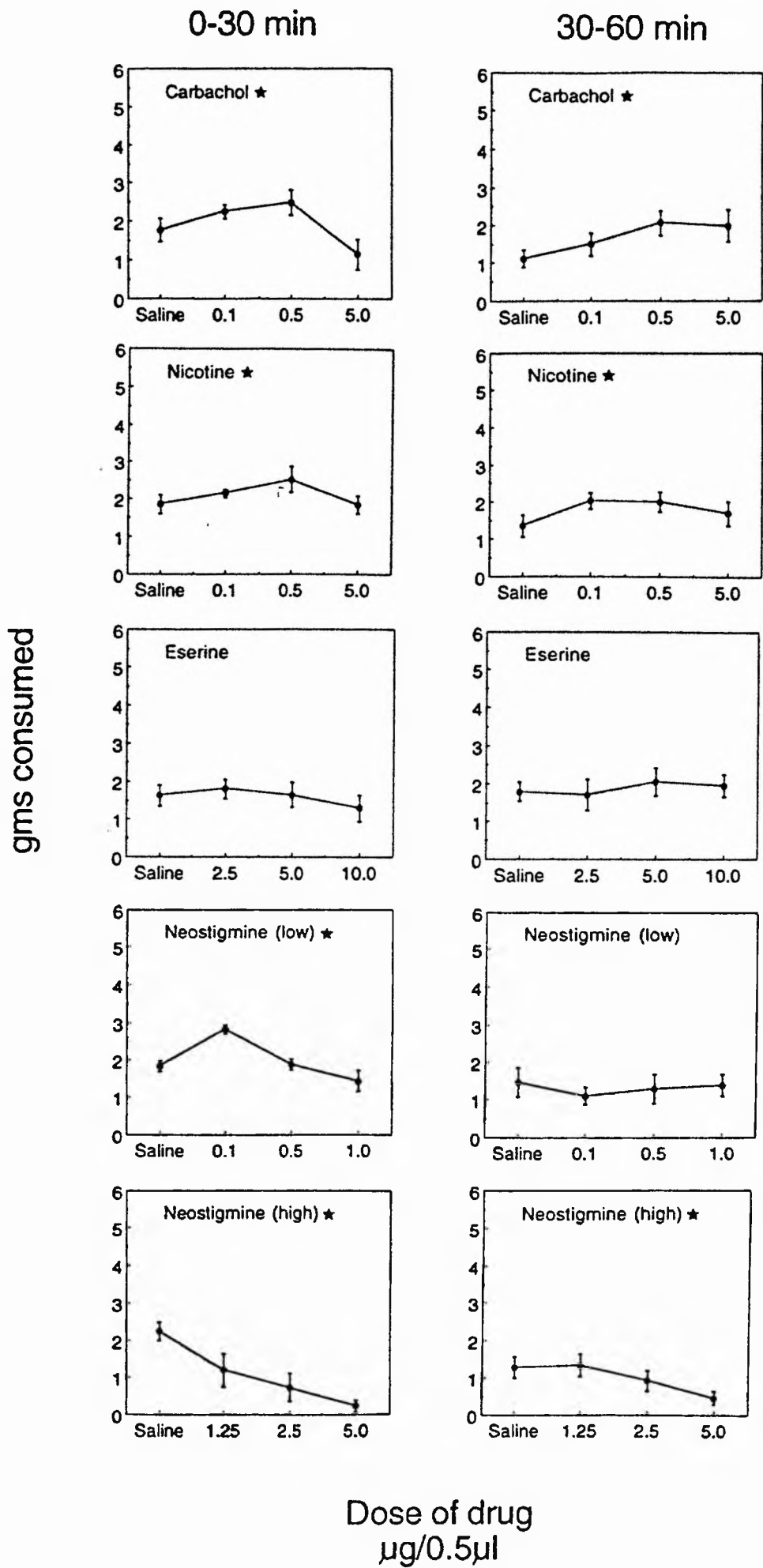


Carbachol

Nicotine

B





main effects of drug ($F=3.36$ $df=4,41$ $p<0.05$), dose ($F=3.74$ $df=3,123$ $p<0.05$) and time ($F=9.37$ $df=2,82$ $p<0.001$), as well as interactions of drug x dose ($F=3.28$ $df=12,123$ $p<0.001$), drug x time ($F=2.07$ $df=8,82$ $p<0.05$) and dose x time ($F=3.20$ $df=6,246$ $p<0.05$). The three way interaction drug x dose x time was not significant ($F=1.02$ $df=24,246$ $p=0.437$).

Further analysis was carried out to discover which drug was producing a significant dose effect over which post-injection period (Figure 6:2). Analysis of the consumption of macaroni following carbachol and nicotine revealed significant main effects of dose (carbachol: $F=4.77$ $df=3,30$ $p<0.01$; nicotine: $F=3.52$ $df=3,27$ $p<0.05$) but no effect of time (carbachol: $F=1.98$ $df=1,10$; nicotine $F=3.32$ $df=1,9$) and no dose x time interaction (carbachol: $F=2.59$ $df=3,30$; nicotine: $F=0.29$ $df=3,27$). The low dose range of neostigmine produced a main effect of dose that approached significance ($F=3.22$ $df=3,15$ $p=0.053$), no main effect of time ($F=5.60$ $df=1,5$) but a significant dose x time interaction ($F=5.12$ $df=3,15$ $p<0.02$). The high dose range of neostigmine showed a significant effect of dose on macaroni consumption ($F=6.02$ $df=3,21$ $p<0.005$), no main effect of time ($F=0.75$ $df=1,7$) but a significant dose x time interaction ($F=4.36$ $df=3,21$ $p<0.02$). Eserine showed no effect of dose ($F=0.38$ $df=3,30$), time ($F=1.09$ $df=1,10$) and no interaction ($F=0.63$ $df=3,30$). However, in every rat tested there was at least one dose of eserine which stimulated more eating than control, although the functional doses and time-period post-injection varied considerably between rats (Table 6:2). Therefore, even although no other cholinergic agent required such an analysis, it might have been more appropriate to use a "best dose" analysis for eserine: to select the dose which stimulated feeding most in individual rats and compare the mean of these values to saline values. Indeed, a "best dose" analysis for carbachol, nicotine or neostigmine would have been unlikely to change any of the conclusions relating to these drugs.

Table 6:2

The intake of dry macaroni by individual rats in the eserine-injected group over 0-30 min and 0-60 min. Every rat ate more macaroni following at least one dose of eserine than following saline in at least one time period (marked in bold and italic print).

0-30 min		Saline	2.5	5.0	10.0
Rat	1	3.7	3.2	3.7	0.7
	2	2.3	2.9	2.2	2.0
	3	0.4	0.8	1.8	0.3
	4	2.1	1.8	0.0	0.8
	5	1.5	0.7	1.6	3.6
	6	1.6	0.9	2.4	0.7
	7	1.1	1.8	2.0	2.4
	8	2.2	2.5	2.1	0.8
	9	1.4	1.8	1.4	2.6
	10	0.4	1.8	0.6	0.0
	11	1.2	1.6	0.3	0.4
0-60 min		Saline	2.5	5.0	10.0
Rat	1	5.5	6.0	6.2	3.4
	2	3.4	3.8	3.8	4.9
	3	3.6	4.7	5.0	2.4
	4	2.1	2.3	3.4	2.1
	5	4.0	4.9	5.3	7.0
	6	3.3	2.3	3.7	2.0
	7	2.9	3.4	4.5	3.6
	8	4.5	3.6	4.6	3.2
	9	3.8	2.8	2.0	3.8
	10	1.5	1.8	0.6	2.6
	11	3.0	2.9	1.5	0.9

Post-hoc analysis revealed significant effects of 0.5 μg carbachol and nicotine over saline when collapsed over time ($p < 0.01$ and $p < 0.05$ respectively). The dose \times time interaction in response to low doses of neostigmine revealed a significant difference between saline and 0.1 μg over 0-30 min ($p < 0.05$). The effect of 0.1 μg neostigmine was greater over the first 30 min than 0.5 μg and 1.0 μg ($p < 0.05$ and $p < 0.01$ respectively), and greater than 0.1 μg over the second 30 min ($p < 0.01$). Within the higher dose range of neostigmine, there was a significant decrease in feeding after 5.0 μg neostigmine compared to saline ($p < 0.05$) when collapsed over time. Therefore only carbachol, nicotine and low doses of neostigmine were able to stimulate feeding of dry macaroni dose-dependently in satiated rats and so the eserine and high neostigmine groups were discarded from the following analyses of different parameters of feeding behaviour.

The mean scores for latency to feed are shown in Table 6:3. There was no significant main effect of dose on latency to feed ($F = 2.18$ $df = 3, 72$) although there was a main effect of drug ($F = 4.97$ $df = 2, 24$ $p < 0.05$); *post hoc* analysis revealed a decrease in latency to feed in response to nicotine compared to carbachol and neostigmine (both $p < 0.05$). Other parameters of eating measured during the first 30 and 60 min are presented in Tables 6:4 and 6:5 respectively. Over the first 30 min of feeding post-injection, there was no main effect of drug on duration of feeding ($F = 0.33$ $df = 2, 24$), number of bouts ($F = 1.24$ $df = 2, 24$), mean bout length ($F = 0.26$ $df = 2, 24$) or rate of feeding ($F = 0.87$ $df = 2, 24$). However, there were main effects of dose on duration of feeding and rate of feeding ($F = 5.20$ $df = 3, 72$ $p < 0.005$ and $F = 2.74$ $df = 3, 72$ $p < 0.05$). Both 0.1 μg and 0.5 μg of each drug significantly increased feeding duration over the highest dose of each drug (5.0 μg carbachol and nicotine; 1.0 μg neostigmine) (both $p < 0.05$) and at 0.5 μg the feeding rate was significantly higher than at the highest drug doses ($p < 0.05$). For mean bout length there was a significant main effect of dose ($F = 3.22$ $df = 3, 72$ $p < 0.05$) and the drug \times dose interaction approached significance ($F = 2.21$ $df = 6, 72$ $p = 0.051$). *Post hoc*

Table 6:3

Latency to feed (min) following unilateral microinjections of various doses ($\mu\text{g}/0.5\ \mu\text{l}$) of cholinergic substances and saline control into rat substantia nigra. Rats which had received nicotine were significantly quicker in the initiation of the first bout of feeding.

	0.0	0.1	0.5	5.0
Carbachol	7.17 ± 2.83	5.81 ± 0.71	6.74 ± 1.34	19.93 ± 4.44
Nicotine *	4.40 ± 0.81	3.60 ± 0.68	3.44 ± 0.70	4.11 ± 1.03
	0.0	0.1	0.5	1.0
Neostigmine (low)	10.26 ± 3.63	7.33 ± 2.10	5.59 ± 1.04	12.50 ± 4.92

Table 6:4

Parameters of feeding measured during 30 min free access to food following unilateral microinjections of various doses of cholinergic drugs and saline control into rat substantia nigra. The drugs did not induce significantly different effects on these parameters although there were effects of dose collapsed over drug (*) (see text for details).

	Duration of feeding(min)	Number of bouts	Mean bout length(min)	Rate of feeding(g/min)
<u>Carbachol</u>				
0.0	10.17 ±1.55	3.09 ±0.48	4.09 ±1.02	0.147 ±0.017
0.1	13.18 * ±1.74	3.73 ±0.27	3.86 ±0.41	0.174 ±0.016
0.5	14.81 * ±1.73	3.45 ±0.39	5.75 ±1.70	0.176 * ±0.012
5.0	7.04 ±2.02	2.54 ±0.74	1.80 * ±0.46	0.174 ±0.047
<u>Nicotine</u>				
0.0	10.10 ±1.87	3.30 ±0.52	3.33 ±0.58	0.210 ±0.02
0.1	11.94 * ±0.63	4.10 ±0.55	3.38 ±0.39	0.182 ±0.011
0.5	13.95 * ±2.88	4.00 ±0.47	3.56 ±0.57	0.195 * ±0.031
5.0	10.78 ±1.48	3.40 ±0.43	3.34 * ±0.51	0.182 ±0.019
<u>Neostigmine (low)</u>				
0.0	11.96 ±2.14	3.00 ±0.58	4.11 ±0.58	0.175 ±0.031
0.1	17.49 * ±1.89	3.50 ±0.34	5.25 ±0.74	0.170 ±0.016
0.5	11.64 * ±2.57	3.83 ±0.60	2.89 ±0.38	0.155 * ±0.013
1.0	9.68 ±3.25	2.50 ±0.76	3.15 * ±0.88	0.140 ±0.033

Table 6:5

Parameters of feeding measured during 60 min free access to food following unilateral microinjections of various doses of cholinergic drugs and saline control into rat substantia nigra. Nicotine stimulated more bouts and higher rates of feeding than neostigmine (see text for details).

	Duration of Feeding(min)	Number of Bouts	Mean Bout Length(min)	Rate of Feeding(g/min)
<u>Carbachol</u>				
0.0	19.12 ±2.63	5.18 ±0.71	4.00 ±0.47	0.152 ±0.011
0.1	24.39 ±2.46	6.18 ±0.48	3.96 ±0.42	0.155 ±0.013
0.5	26.78 ±1.70	6.09 ±0.59	5.00 ±0.79	0.171 ±0.011
5.0	19.22 ±3.03	5.18 ±0.77	4.42 ±0.66	0.175 ±0.024
<u>Nicotine</u>				
0.0	18.84 ±3.33	6.10 ±0.92	3.07 ±0.35	0.188 ±0.018
0.1	24.15 ±1.42	7.80 ±0.71	3.40 ±0.39	0.176 ±0.012
0.5	24.99 ±3.87	7.80 ±0.57	3.19 ±0.41	0.201 ±0.019
5.0	20.55 ±2.28	6.10 ±0.57	3.67 ±0.51	0.184 ±0.021
<u>Neostigmine (low)</u>				
0.0	24.80 ±3.69	5.17 ±0.60	4.91 ±0.65	0.141 ±0.012
0.1	27.10 ±3.49	5.17 ±0.60	5.66 ±1.01	0.153 ±0.012
0.5	23.08 ±4.75	5.83 ±0.95	3.62 ±0.60	0.139 ±0.010
1.0	20.78 ±3.95	4.50 ±0.99	5.06 ±0.77	0.141 ±0.013

testing revealed that mean bout length was significantly decreased at the highest doses although the fact that the interaction approached significance suggests that this effect mainly occurred because of the very short bout lengths observed at the highest dose of carbachol (5.0 μ g). Over a 60 min post-injection period (Table 6:5) there were effects of drug on mean bout length and rate of feeding ($F=5.36$ $df=2,24$ $p<0.05$ and $F=3.33$ $df=2,24$ $p=0.05$); *post hoc* testing revealed that nicotine stimulated more bouts and higher rates of feeding than neostigmine (both $p<0.05$). This is presumably related to the different feeding time courses observed for these drugs, with nicotine driving feeding-related activity for 60 min while neostigmine had its main effects only during the first 30 min.

Consumption of lab chow was negligible, occurring on only 8/456 tests and always following stimulation by the highest doses of neostigmine and carbachol. No rat ever ate more than 0.2 g lab chow in 30 min following microinjection. Table 6:6 shows the mean intake of tap water following microinjection. The amount of tap water drunk showed a main effect of drug ($F=3.49$ $df=4,41$ $p<0.05$) but there were no effects of dose for any drug group (carbachol: $F=0.03$ $df=3,30$; nicotine $F=0.94$ $df=3,27$; eserine $F=0.70$ $df=3,30$; low neostigmine: $F=1.23$ $df=3,15$; high neostigmine: $F=0.53$ $df=3,21$). The effect of drug was found to be the product of an increase in drinking in the high dose neostigmine group ($p<0.05$).

Behavioural analysis

Examination of still/asleep, locomotor, rearing, sniffing, gnawing of polystyrene chips and grooming responses was made (Table 6:7). The only differences between the drugs occurred with the highest dose of carbachol and the higher doses of neostigmine. No dose of nicotine, eserine, or lower doses of neostigmine had effects different to saline in any behavioural category. The highest dose of carbachol increased rearing in 5/11 rats and the highest doses of neostigmine increased rearing and sniffing in > 50% rats. (This proportion was not increased by

Table 6.6

The mean intake of tap water during each 30 min and during 60 min following unilateral microinjection of various doses ($\mu\text{g}/0.5 \mu\text{l}$) of cholinergic substances and saline control into rat substantia nigra. Rats in the neostigmine (high) group drank significantly more water than those in the neostigmine (low) or eserine groups (see text for details).

	Dose	0-30 min	30-60 min	0-60 min
Carbachol	0.0	1.80 ± 0.24	1.44 ± 0.17	3.24 ± 0.34
	0.1	1.68 ± 0.22	1.58 ± 0.31	3.26 ± 0.41
	0.5	1.68 ± 0.30	1.72 ± 0.29	3.40 ± 0.39
	5.0	2.02 ± 0.74	1.26 ± 0.28	3.28 ± 0.86
Nicotine	0.0	1.72 ± 0.15	1.82 ± 0.31	3.54 ± 0.41
	0.1	2.21 ± 0.23	1.61 ± 0.23	3.82 ± 0.39
	0.5	1.66 ± 0.16	1.55 ± 0.09	3.21 ± 0.15
	5.0	1.87 ± 0.24	1.49 ± 0.29	3.36 ± 0.44
Eserine	0.0	1.71 ± 0.25	1.59 ± 0.22	3.30 ± 0.31
	2.5	1.61 ± 0.11	1.56 ± 0.15	3.17 ± 0.23
	5.0	1.89 ± 0.14	1.74 ± 0.36	3.63 ± 0.42
	10.0	1.54 ± 0.20	1.66 ± 0.21	3.20 ± 0.35
Neostigmine (low)	0.0	1.38 ± 0.28	1.13 ± 0.28	2.51 ± 0.40
	0.1	1.62 ± 0.46	1.35 ± 0.27	2.97 ± 0.69
	0.5	1.68 ± 0.26	1.28 ± 0.17	2.96 ± 0.37
	1.0	1.37 ± 0.26	1.03 ± 0.19	2.40 ± 0.37
Neostigmine (high)	0.0	2.62 ± 0.35	2.21 ± 0.22	4.83 ± 0.53
	1.25	2.44 ± 0.33	2.15 ± 0.25	4.59 ± 0.55
	2.5	3.22 ± 0.37	1.65 ± 0.14	4.87 ± 0.41
	5.0	2.54 ± 0.33	1.83 ± 0.24	4.37 ± 0.54

Table 6:7

Measures of activities scored on the Fray checklist during 0-30 and 0-60 min following microinjection. Means and standard deviations were calculated for each behaviour using saline values from all 46 rats. Shown below are the number of rats in a given group who scored greater than the [mean value + (2 x standard deviation)] for a given dose. Figures are highlighted in bold and italic print where they approach 50% or are > 50% of rats in that group.

	Number of rats meeting criterion					
	0-30 min			0-60 min		
Dose ($\mu\text{g}/0.5\mu\text{l}$)	0.1	0.5	5.0	0.1	0.5	5.0
<u>Carbachol (n=11)</u>						
Locomotion	2	2	2	0	1	0
Still/asleep	0	0	2	0	0	1
Rearing	0	0	5	0	0	2
Sniffing	0	1	0	1	1	1
Gnawing	0	1	0	0	0	0
<u>Nicotine (n=10)</u>						
Locomotion	0	0	0	0	1	1
Still/asleep	1	0	1	0	0	0
Rearing	0	1	1	0	1	1
Sniffing	0	1	0	1	2	1
Gnawing	0	0	1	0	0	1
<u>Eserine (n=11)</u>						
Dose ($\mu\text{g}/0.5\mu\text{l}$)	2.5	5.0	10.0	2.5	5.0	10.0
Locomotion	3	2	0	2	3	0
Still/asleep	1	1	3	0	1	1
Rearing	0	0	1	0	0	2
Sniffing	1	0	0	1	0	1
Gnawing	0	1	0	0	0	0

Table 6:7 (continued)

	Number of animals meeting criterion					
	0-30 min			0-60 min		
Dose ($\mu\text{g}/0.5\mu\text{l}$)	0.1	0.5	1.0	0.1	0.5	1.0
<u>Neostigmine (Low) (n=6)</u>						
Locomotion	0	0	0	0	0	0
Still/asleep	0	0	0	0	0	0
Rearing	0	0	1	0	1	3
Sniffing	0	0	2	0	1	2
Gnawing	1	0	0	0	0	0
Dose ($\mu\text{g}/0.5\mu\text{l}$)	1.25	2.5	5.0	1.25	2.5	5.0
<u>Neostigmine (High) (n=8)</u>						
Locomotion	2	2	0	1	1	1
Still/asleep	1	3	4	0	2	1
Rearing	5	2	3	4	4	3
Sniffing	5	3	1	6	2	5
Gnawing	0	0	0	0	0	0

relaxing the criterion to $> \text{mean} + 1 \text{ SD}$ above control: 2 rats showed no difference in their responses to high doses of neostigmine and saline.) Other forms of behaviour observed included ipsilateral turning and scrabbling with the forepaws and cage-bar gnawing following high doses of neostigmine. It should also be noted that at $5.0 \mu\text{g}$ neostigmine, rats were significantly more likely to be rated in the still/asleep category during the first 30 min. In fact these rats were never asleep, but instead appeared rigidly still and alert, as if in the form of a catatonic seizure. Seizure-related activities ("wet dog shaking" and convulsions) were also seen following $5.0 \mu\text{g}$ carbachol and two rats not included in the analyses died following convulsions after $5.0 \mu\text{g}$ neostigmine.

Discussion

Microinjections of carbachol, nicotine and low doses of neostigmine into the anterior SN elicited dose-dependent increases in the consumption of palatable food. This cholinergic stimulation of feeding was associated with an extension of the duration of feeding, longer feeding bouts and an increased rate of eating. Nicotine also decreased the latency to feed compared with carbachol and neostigmine, although this result must be viewed with caution because the latencies to feed following saline were so markedly different. The time courses of feeding also differed: carbachol and nicotine elicited significant dose-dependent increases in feeding over 60 min post-injection while neostigmine increased feeding only over the first 30 min. At higher doses in some rats, carbachol and neostigmine increased the frequency of sniffing and rearing although these activities had no apparent goal. No specific doses of eserine increased feeding, although feeding activity was increased in each individual rat following at least one dose and during at least one time period post-injection. Nicotine, eserine and the lower doses of carbachol and neostigmine did not significantly affect activities other than eating.

Cholinergic stimulation of SN by direct cholinergic agonists

In the present study, the behavioural effects of nicotine and carbachol were almost indistinguishable. Perhaps the most important aspect to note, however, was the inability to initiate convulsions with nicotine. It may be that the doses of nicotine used here were not high enough to instigate seizure activity. However, it is more appealing to speculate that muscarinic stimulation is specifically required for seizure activity. It has previously been suggested that the SN has a direct role in seizure initiation, following demonstration of seizure development after injecting the GABA antagonist picrotoxin into SN (Arnt and Scheel-Krüger, 1979). Links between muscarinic receptor activation and seizures have also been tracked to the SN. The susceptibility to seizures induction by the predominantly muscarinic agonist, pilocarpine, is reduced by increasing levels of GABA in the SNr (Turski *et al.*, 1986). This not only highlights the muscarinic association with seizures, but also emphasises the close functional links between SNc and SNr previously described in Chapter 2.

It is likely that there are other subtle distinctions between the effects of muscarinic and nicotinic stimulation at the level of the SNc. Indeed in the VTA muscarinic and nicotinic receptors have been dissociated on the basis of their different effects on discriminative cues and intracranial self-stimulation (Druhan *et al.*, 1989). While the muscarinic agonists pilocarpine and RS-86 facilitated electrical brain-stimulation cues, nicotine had no effect. On the other hand, nicotine and the muscarinic receptor antagonist scopolamine increased rates of intracranial self-stimulation, while pilocarpine depressed self-stimulation rates. Although from one perspective this might suggest that cholinergic stimulation of the VTA activates separable cue and reward processes, these are likely to be complementary as there is evidence to suggest that DA is involved in both (Druhan *et al.*, 1987; Fibiger *et al.*, 1987). Indeed it would be rather remarkable if the functions of muscarinic and nicotinic receptors in VTA or in SN were not complementary, given that their

normal actions would be carried out in parallel following the release of ACh. Addition of carbachol to doses of nicotine injected into SN is known to be additive where palatable food intake is concerned (Parker and Winn, 1992) and examination of other forms of behaviour (for example, conditioned feeding) would probably reveal similar interactions between nicotinic and muscarinic activation there.

Cholinergic stimulation of SN by anticholinesterases

The effects of neostigmine did not differ in any significant manner from nicotine and carbachol apart from the time profile of feeding behaviour. This is consistent with the assumption that neostigmine-induced blockade of AChE potentiates the action of endogenous ACh, which then acts at receptors on DA-containing SNc neurones. It remains to be determined which cholinergic sub-type is responsible for the effects, although it is most likely to be both (Parker and Winn, 1992). However, the data in this study suggest that anticholinesterases may be erratic in their ability to elicit a feeding response when injected into SN. For instance, although eserine has previously been shown to increase feeding dose-dependently when injected into SN (Winn and Redgrave, 1981), this effect was not replicated here, although there was a dose of eserine which stimulated more eating than control for every rat tested (Table 6:2).

The inconsistent effects of AChE-inhibitors must reflect their mode of action and presumably the greater variability of eserine reflects pharmacological differences between this and neostigmine. In order to ascertain why the effects of eserine appear to be so variable, it is necessary to look at differences in its structure compared to neostigmine and how such differences might relate to AChE inhibition. Although differences in chemical composition between the two molecules have been described, how these affect their pharmacological actions in the central nervous system is not yet well understood.

Neostigmine is a quaternary ammonium compound, is completely ionized in aqueous solution regardless of the pH of the medium and therefore retains its inhibitory potency in any ionic solution. Eserine is a tertiary amine and its degree of ionization is dependent on the pH of the medium. Both eserine and neostigmine act by binding strongly at the anionic site on the cholinesterase enzyme: as they are hydrolysed much less readily than ACh (Myers, 1956) they remain there for a considerably longer time, during which the enzyme cannot hydrolyse ACh. The inhibitors compete with ACh for the anionic site on the enzyme. In theory the configurations of the drugs should be virtually identical at body pH. However there are sufficient structural differences to confer subtle pharmacological variations. As the solution becomes more alkaline the potency of eserine inhibition declines in parallel with its change in configuration (Wilson and Bergmann, 1950), such that in the body at approximately pH 7.4 neostigmine will be slightly more potent. This weakening of ionic attraction also means that eserine is more likely to diffuse away from the injection site, therefore producing relatively transient effects. Furthermore in the periphery, studies have shown that eserine but not neostigmine blocks ACh release (Alderdice, 1979) which, if also true centrally, would be counter-productive in the present experiments.

In the periphery at high doses, neostigmine has been shown to have direct stimulatory effects on postsynaptic receptors in addition to its anticholinesterase activity (Bowman and Rand, 1980, p. 10.36; Goodman Gilman *et al.*, 1980, p. 108). This is presumably due to its structural similarity to ACh. The possibility that the feeding effects of neostigmine were mediated directly at the post-synaptic membrane is however unlikely: it has been shown that while quinolinate lesions placed caudal to SN in the PPTg greatly attenuated the increased efflux of DA in the dorsal striatum which followed intranigral neostigmine, such lesions enhanced the DA-releasing properties of nicotine, presumably through proliferation and increased sensitivity of the postsynaptic receptors on SNc neurones (Blaha and

Winn, 1993). If increased feeding following nigral neostigmine occurred primarily through direct nicotinic receptor stimulation, DA release in the CPu in the PPTg-lesioned rat would be enhanced following both neostigmine and nicotine administration.

The relatively short-lived increase in feeding following neostigmine injection may relate to the secondary actions of AChE in SN. As suggested in Chapter 2, released AChE may affect GABAergic transmission in the SNr. Therefore although neostigmine-induced blockade of AChE would increase levels of ACh to stimulate SNc, the additional implicit manipulation of SNr would compromise the normal neuronal interactions in SN. Increased activation of SNc induces DA release from pars compacta neurones to inhibit further firing via an autoreceptor. Subsequently, recurrent SNc firing is probably controlled by balanced interactions between ACh inputs and GABAergic outflow of motor information. If AChE plays a major role in these interactions then it may not be entirely surprising that the effects of neostigmine are so transitory.

Comparison of general behavioural effects

It has previously been shown that microinjections of carbachol into the anterior SN increase locomotion in the presence of a palatable food (Winn *et al.*, 1983). However, in this study there were no observable differences following any drug in any of the behavioural categories except feeding. Given the different effector routes of the drugs used, it is somewhat surprising that there are no differences in the general behavioural data.

Behavioural effects of SP following AChE inhibition. AChE is known to have peptidase activity towards substance P (SP) (Chubb *et al.*, 1980). Therefore AChE inhibition by eserine and neostigmine would be expected to increase both SP and ACh levels in SN. The behavioural effects of direct SP infusion into SN have been

previously examined (Kelley and Iversen, 1978, 1979). Following extensive habituation in photocell cages, intranigral SP elicited an increase in whole-body locomotor activity, while in the open field the same infusion was expressed behaviourally in the form of stereotyped rearing along the wall with no concomitant increase in locomotion. With subsequent infusions in both circumstances, the response changed to grooming. The pharmacological basis for this phenomenon is unknown, although Kelley and Iversen (1979) suggested that some form of long-term neuronal change may occur which progressively sensitizes (or de-sensitizes) SP receptors.

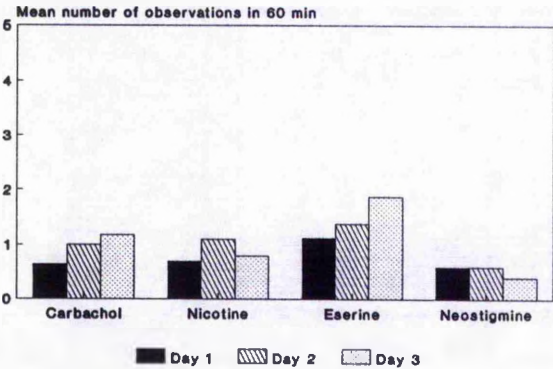
In this study, no increases in locomotion, rearing or grooming were observed (except as part of seizure-type activity at high doses) following either eserine or neostigmine. Indeed the incidence of any behaviours other than feeding was very small and it is likely that the presence of a palatable foodstuff suppressed the expression of other forms of behaviour. However even with the low incidence levels of other behaviours noted in the present study, the sort of neuronal modification postulated by Kelley and Iversen suggests an alternative means of viewing the data. Each rat received the different doses of its particular drug treatment in a random order. If on the first day the most prevalent behaviour other than feeding was rearing/locomotion and on the last day it had changed to grooming, analysis of the data by dose would be likely to mask such an effect. Unfortunately, the low incidence of these behaviours and the limited sampling window used to score them make statistical analysis of the data by day difficult. However, even with such constraints, Figure 6:3 suggests a tenuous trend towards differences in the way that AChE-inhibitors and direct agonists affected the incidence of behaviours other than feeding with repeated injections. Of specific note is the changing profile of behaviours following repeated low doses of neostigmine, particularly in comparison to the profile following carbachol.

Figure 6:3

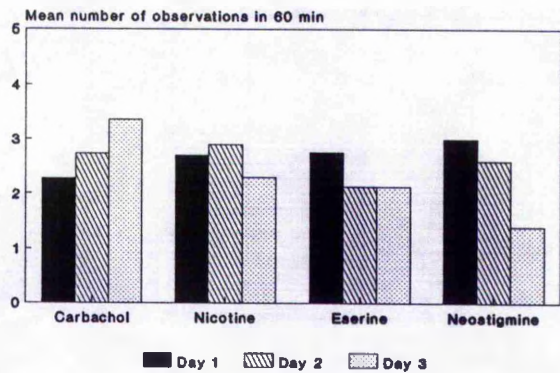
Change in behavioural responses to repeated injections of carbachol, nicotine, eserine or low doses of neostigmine into the rat substantia nigra. Saline data were removed and all remaining data for doses on each day were amalgamated into one group. Bars refer to the mean number of observations in 60 min (total possible = 10) on each day.

Figure 6:3

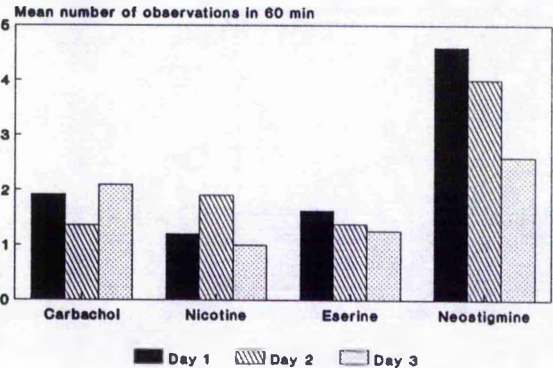
Locomotion



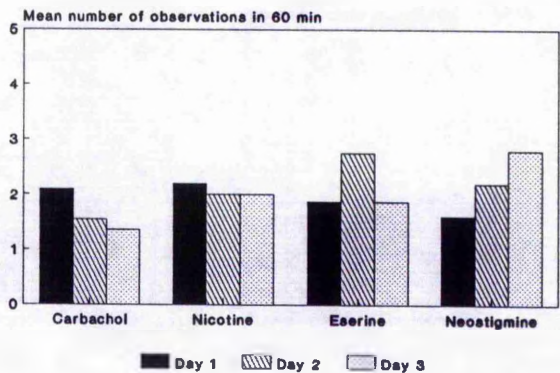
Sniffing



Rearing



Grooming



Cholinergic stimulation of substantia nigra and DA-mediated behaviour

A body of anatomical, electrophysiological, biochemical and behavioural evidence strongly indicates that the effects of cholinergic stimulation of the anterior SN are mediated by nigrostriatal DA-containing neurones (for instance Taha and Redgrave, 1980; Lichtensteiger *et al.*, 1982; Winn *et al.*, 1982; Clarke and Pert, 1985; Bolam *et al.*, 1991; Parker *et al.*, 1991; Blaha and Winn, 1993; see Chapter 2 for details). DA in the dorsal striatum is thought to mediate the behavioural stereotypies produced by high doses of systemic AMPH (Kelly *et al.*, 1975) and the oral motor stereotypies following direct DAergic stimulation of the ventrolateral striatum (Delfs and Kelley, 1990; Kelley *et al.*, 1988). In agreement with previous studies however (Winn *et al.*, 1983; Winn, 1990) no similar pattern of stereotypy was observed following cholinergic stimulation of SN. The high doses of neostigmine did produce increased frequencies of sniffing and rearing, with all but two rats showing frequencies greater than the saline mean + 2SD. However, the relationship between this and the pattern of stereotypy produced by AMPH is not clear. AMPH-treated rats tend to show sniffing, rearing and locomotion (3 on the Creese-Iversen scale [Kelly *et al.*, 1975]), these giving way at higher doses to "head down" sniffing maintained in one location, usually the corner of the cage. Neostigmine-treated rats on the other hand, showed little increase in locomotion and no "head down" sniffing, but did show "wet-dog shaking", convulsions and catatonia which may have more in common with the development of seizures as with stereotypies. A role for the SN in relation to seizure activity was discussed above.

A number of reasons can be advanced to explain the general failure of cholinergic stimulation to induced clear stereotypy: first, the level of stimulation may have been insufficient. This is unlikely to be the case as the highest dose of carbachol used was only just below that needed to consistently promote convulsions (Winn *et al.*, 1983) and the highest doses of neostigmine did produce lethal convulsions,

unless the intrusion of seizures suppressed the development of stereotyped behaviour. Second, it could be argued that a particular combination of nicotinic and muscarinic activation is needed to produce sufficient DA release in the CPu for stereotypy. This also is unlikely to be the case given that neostigmine, potentiating the effects of endogenous ACh, did not produce clear AMPH-like stereotypy. The third explanation is related to this, but in reverse. The precise means by which AMPH releases DA is still the subject of debate, but recent *in vitro* studies suggest that AMPH is a potent competitor for the uptake carrier and is transported intraneuronally, after which the carrier reverses, preferentially releasing DA from the newly synthesized pool in the cytoplasm (Butcher *et al.*, 1988). If this is the case it is likely that AMPH is able to release much more DA than can be achieved by the generation of action potentials from the SNc alone. Stimulation of SNc is likely to induce physiological levels of DA release and these appear not to be sufficient for the induction of AMPH-like stereotypy. Recognition of such factors is important for determining the most appropriate means of experimentally stimulating dorsal striatal DA activity.

However it should be pointed out that injections of AMPH or APO directly into the CPu have failed themselves to elicit the types of stereotypies typically observed following the systemic drug treatments (Robbins *et al.*, 1990). For instance, bilateral injections of 10 - 100 μ g APO into the CPu did not elicit gnawing behaviour although some "head-down" sniffing was observed at the higher doses. Similarly, bilateral AMPH injections of 3 - 30 μ g into the CPu did not elicit typical AMPH stereotypy and exceptionally high doses (80 μ g and 160 μ g) only generated slight increases in locomotion, sniffing and head movements. Although these data appear to contradict the lesion data which implicate the CPu as a crucial structure in the production of stereotypies (Kelly *et al.*, 1975), they also support the hypothesis that the CPu is necessary for the production of stereotyped behaviour, but is not by itself sufficient. Such a hypothesis is given further support by the

present data, where even the most powerful levels of cholinergic stimulation failed to elicit AMPH-like stereotypies.

Conclusions

The data presented in this Chapter demonstrate that:

1. Intranigral carbachol, nicotine or neostigmine can elicit dose-dependent feeding of palatable food from satiated rats, demonstrating that this behaviour can be mediated by muscarinic and nicotinic receptors.
2. Eserine increased feeding of palatable food in satiated rats, although the effect of different doses was inconsistent across subjects. This may be due to the variable potency of eserine with pH.
3. Just as intranigral carbachol could not elicit general behavioural activation (Winn *et al.*, 1983), no behaviours other than feeding were elicited by nicotine, eserine or lower doses of neostigmine. This emphasises the behavioural specificity of cholinergic stimulation.
4. High doses of carbachol and neostigmine, but not nicotine, injected into SN induced seizure activity. This highlights the links between the SN and seizure activity and may implicate muscarinic, rather than nicotinic, mechanisms in this.

7. Control of striatal dopamine by cholinergic neurones in the pons: effects on feeding and drinking of cholinergic antagonism in the substantia nigra

Introduction

The previous study demonstrated that stimulation of SN by neostigmine methyl sulphate (NEO) dose-dependently increased feeding in satiated rats. This consummatory activation is thought to occur because NEO competes for the anionic site on the cholinesterase enzyme, preventing hydrolysis of the endogenous ACh and boosting its concentration in the synaptic cleft to produce a stimulatory action on nicotinic and muscarinic receptors located postsynaptically on nigrostriatal DA neurones. The increased feeding following NEO should therefore be attenuated by cholinergic receptor antagonists applied to SN.

Following a series of experiments which investigated AChE release from DAergic somata and dendrites in the SNc, Greenfield and colleagues suggested that AChE may have novel functions there (Greenfield, 1984). Given this possibility, it is conceivable that the behavioural consequences of NEO injections into SN are not straightforwardly due to its cholinergic actions. For instance, NEO may also block actions of released AChE on SNr neurones and this blockade may have secondary potentiating or attenuating effects on feeding. While it is likely that such complementary actions of cholinesterase inhibition on the feeding response would normally be overshadowed by the cholinergic excitation, co-injection of a cholinergic receptor antagonist might tease out possible underlying influences.

Therefore, in this study the effects of 2 cholinergic antagonists, the nicotinic receptor antagonist mecamylamine hydrogen chloride and the muscarinic receptor antagonist atropine sulphate, were considered. The study consisted of 2 separately

conducted experiments: in the first experiment the antagonists were individually administered to the SN in the absence of NEO to verify that they themselves had no specific effects there; in the second experiment each antagonist was injected into the SN in conjunction with the active dose of NEO from the previous study (0.1 μg / 0.5 μl) once the placement of the injection needle for each rat had been validated by recording the feeding response following saline and 0.1 μg NEO.

Methods

Animals

1) *Receptor antagonists alone.* 16 male Lister hooded rats with body weight at the time of surgery 315.6 (\pm 18.16 [SD])g.

2) *Neostigmine with receptor antagonists.* 20 male Lister hooded rats with body weight at the time of surgery 305.5 (\pm 9.25 [SD])g.

Surgery

Rats were anaesthetised with ketamine and xylazine and implanted unilaterally with a stainless steel guide cannula positioned above the substantia nigra.

Intracranial microinjection

1) *Receptor antagonists alone.* Rats were arbitrarily assigned to one of two drug groups. Each group was given 4 injections to the SN of saline vehicle and either atropine sulphate (0.1, 0.5, 1.0 μg) or mecamlamine hydrogen chloride (0.05, 0.1, 0.5 μg). These doses were in the same nmolar range as the dose of NEO which previously stimulated feeding.

2) *Neostigmine with receptor antagonists.* Consummatory responses were initially probed by comparing the food and water intake of each rat following injections of saline and 0.1 μg / 0.5 μl neostigmine methyl sulphate. Rats which increased their

feeding response following the NEO injection were arbitrarily assigned to one of two drug groups. Each group was given a further 4 injections to the SN of 0.5 μ g NEO with either atropine sulphate (0.1, 0.5, 1.0 μ g) or mecamylamine hydrogen chloride (0.05, 0.1, 0.5 μ g). Following these injections, feeding responses were re-examined after an injection of NEO (0.1 μ g / 0.5 μ l).

Behavioural testing procedure

Rats were observed in the unconditioned feeding paradigm (see General Methods). During the habituation and test sessions all rats had free access to weighed amounts of tap water, normal lab chow, dry macaroni (580 Kj/100 g, 4.5 g protein/100 g) and polystyrene packing chips. Rats were habituated to the unconditioned feeding test environment for 60 min before and after sham-microinjection on consecutive days until feeding before and after the injection procedure was stable. The testing phase then began with food and water consumption measured for 60 min before and after microinjection.

Statistical Analysis

1) *Receptor antagonists alone.* Food and water intake data were analysed parametrically by ANOVA where type and dose of antagonist were the dependent variables.

2) *Neostigmine with receptor antagonists.* The increased feeding following NEO compared to saline was analysed parametrically with a t-test. Food intake following NEO in conjunction with the antagonists was analysed by parametrically by ANOVA where type of antagonist and dose were the dependent variables. A "best dose" analysis was also carried out (t-test) by comparing the mean feeding response following NEO alone with the mean of the lowest feeding responses following NEO in conjunction with an antagonist.

Results

Histological analysis

Figure 7:1 presents representative sections indicating injection placements for both experiments. Following histological examination, 11 rats receiving receptor antagonists alone were found to have injection sites in or immediately adjacent to SN while 5 rats in this experiment had misplaced cannulae and were discarded from the analyses. The 10 rats which increased their feeding following NEO compared to saline in the second experiment all had injection sites in SN. 10 rats probed with NEO in this experiment did not increase their feeding activity at this dose compared to saline. In these cases, the injection cannula was lowered and consumption following saline and NEO was probed once again. However, these subsequent injection sites were found to be in the most ventral part of posterior SN (or in some cases even out of the base of the brain), suggesting that the original injection site had actually been in the appropriate stereotaxic position.

Eating and drinking in response to cholinergic receptor antagonism in SN

1) *Receptor antagonists alone.* Table 7:1 lists the mean intake of dry macaroni and tap water during the 60 min following microinjection. The amount of macaroni consumed post-injection revealed no significant main effects of antagonist ($F=0.11$ $df=1,9$) or dose ($F=0.88$ $df=3,27$) and there was no significant antagonist x dose interaction ($F=1.19$ $df=3,27$). Similarly, there were no effects of antagonist or dose on the amount of tap water drunk ($F=0.36$ $df=1,9$ and $F=0.89$ $df=3,27$ respectively) and again the drug x dose interaction was not significant ($F=1.49$ $df=3,27$).

2) *Neostigmine with receptor antagonists.* A summary of the data collected in each group is illustrated in Figure 7:2. Allocation of rats to their experimental groups was carried out after an increased feeding response was achieved following NEO compared to saline. The saline data were demonstrated to be significantly different

Figure 7:1

Representative sections, redrawn from the atlas of Paxinos and Watson (1982) showing placements. The placements of cannulae delivering the different drugs (ATR: atropine; MEC: mecamlamine) are shown separately, collapsed onto right or left hemispheres (open circles: antagonist only, nigral injections; open triangles: antagonist only, misplaced injections; closed circles: 0.1 μ g / 0.5 μ l neostigmine in conjunction with antagonist, functional sites).

ATR

MEC

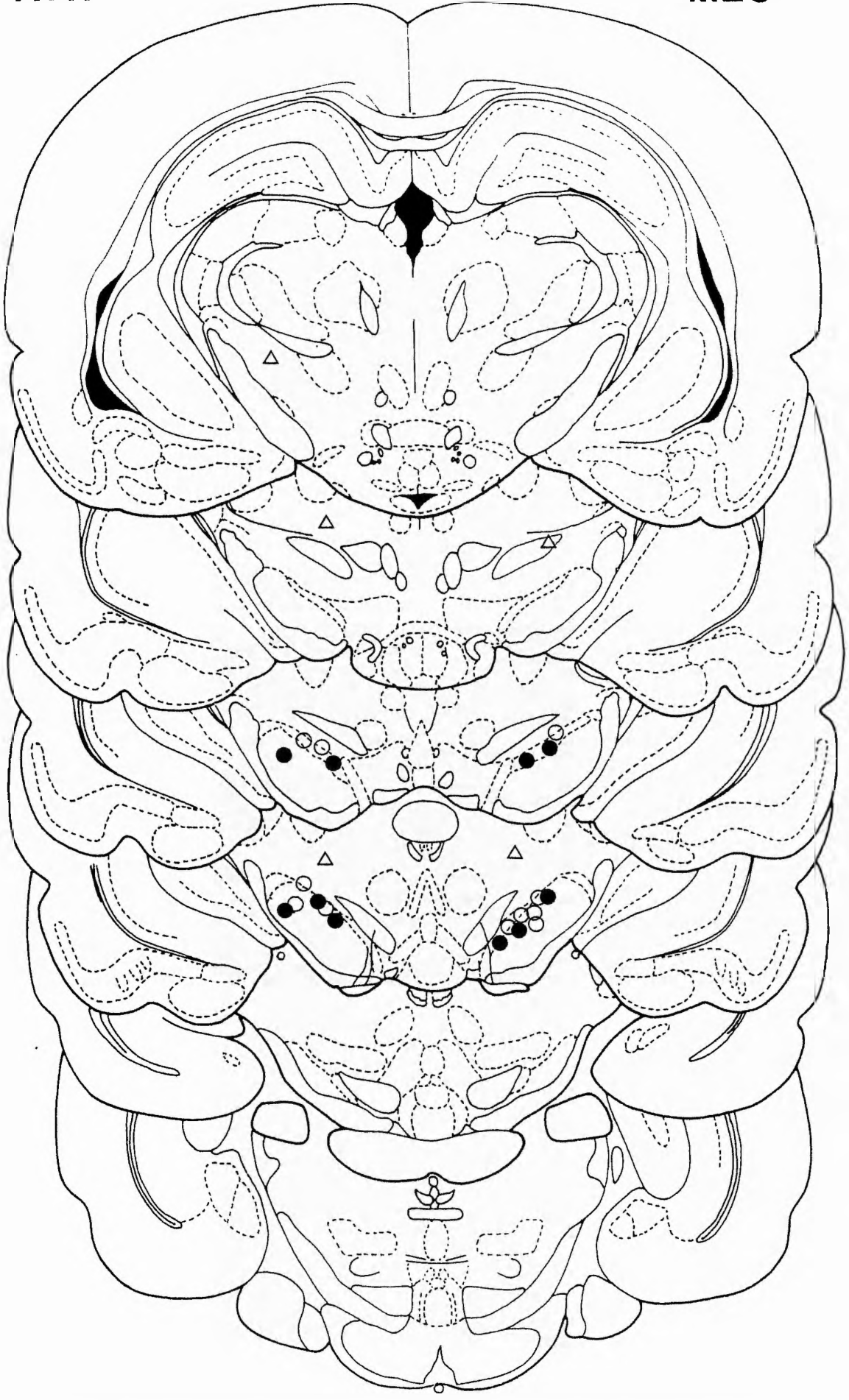


Table 7:1

The mean intake of dry macaroni and tap water during 60 min following unilateral microinjection of various doses ($\mu\text{g} / 0.5\mu\text{l}$) of cholinergic antagonists or saline vehicle into rat substantia nigra. There were no significant differences in food or water intake.

Atropine	0.0	0.1	0.5	1.0
Food	2.08 ± 1.04	2.28 ± 0.78	1.86 ± 1.06	1.92 ± 0.83
Water	3.02 ± 0.47	2.66 ± 0.39	2.14 ± 0.26	2.60 ± 0.13
<hr/>				
Mecamylamine	0.0	0.05	0.1	0.5
Food	3.25 ± 0.58	2.13 ± 0.39	2.05 ± 0.60	1.87 ± 0.49
Water	2.47 ± 0.26	2.70 ± 0.27	2.68 ± 0.29	2.03 ± 0.33

to the first NEO injection ($t=-6.50$ $df=9$ $p<0.001$) but not to the second NEO (final) injection ($t=-1.15$ $df=9$), although the 2 NEO injections were not significantly different to each other ($t=0.71$ $df=9$) (Figure 7:3). The feeding response following saline was also significantly different to the mean of the responses following NEO ($t=-2.80$ $df=9$ $p<0.05$) and therefore the NEO data were collapsed for all subsequent comparisons. The groups were balanced so that there were no significant group differences between saline- and NEO-induced feeding responses ($F=0.14$ $df=1,8$). 3-way ANOVA demonstrated that there were no differences in the way each antagonist affected this increased feeding response ($F=0.63$ $df=1,8$) and there was no effect of dose ($F=0.07$ $df=2,16$) or an antagonist by dose interaction ($F=0.20$ $df=2,16$). Therefore for the following analysis the data from the 2 antagonist groups were collapsed.

Given the inconsistent effects obtained from NEO injections to the SN and the differences in magnitude of the feeding responses obtained from individual rats, an analysis of the antagonist data by using the "best dose" for each rat seemed appropriate. The dose of antagonist at which the greatest attenuation of NEO-stimulated food intake occurred was designated the "best dose" for that rat. It can be seen from Figure 7:4 that the mean best dose NEO+antagonist significantly attenuated food intake compared to the effects of NEO alone ($t=3.21$ $df=9$ $p<0.001$) and the response was not significantly different to food intake following saline ($t=0.59$ $df=9$).

Discussion

These data demonstrate that microinjection of either atropine or mecamylamine alone into the anterior SN does not significantly alter the baseline feeding or drinking activity measured following injections of saline. However increased feeding following NEO injections ($0.1 \mu\text{g}$ / $0.5 \mu\text{g}$) to the SN can be attenuated by

Figure 7:2

Mean amounts of dry macaroni consumed during 60 min following unilateral microinjection of saline, 0.1 μ g neostigmine and neostigmine in conjunction with various doses (μ g / 0.5 μ l) of cholinergic antagonists into the rat substantia nigra.

Figure 7:3

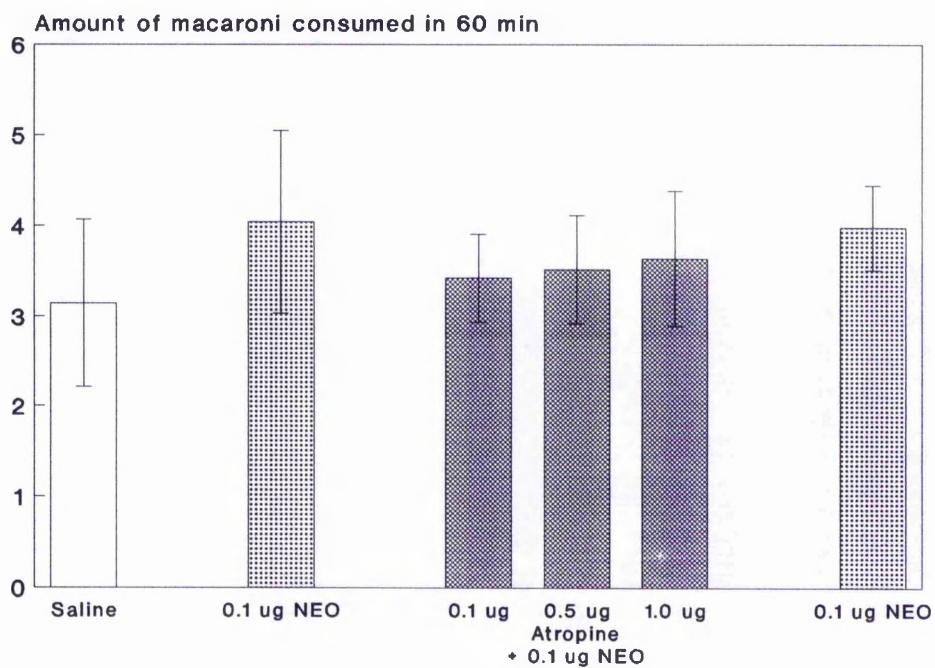
Feeding response following saline and the significant increase in this response following neostigmine injection to the SN before, but not after, injections of cholinergic antagonists (** $p < 0.001$). The mean response following neostigmine injection was also significantly increased compared to saline responses (* $p < 0.05$). Only 50% of rats tested increased their feeding responses by greater than 0.5 g following intranigral neostigmine compared to saline, suggesting inconsistencies in dose-effects for this drug.

Figure 7:4

The mean intake of macaroni in 60 min following saline, neostigmine alone, or neostigmine in conjunction with the "best dose" of receptor antagonist (* $p < 0.001$ compared to neostigmine alone).

Atropine

Figure 7:2



Mecamylamine

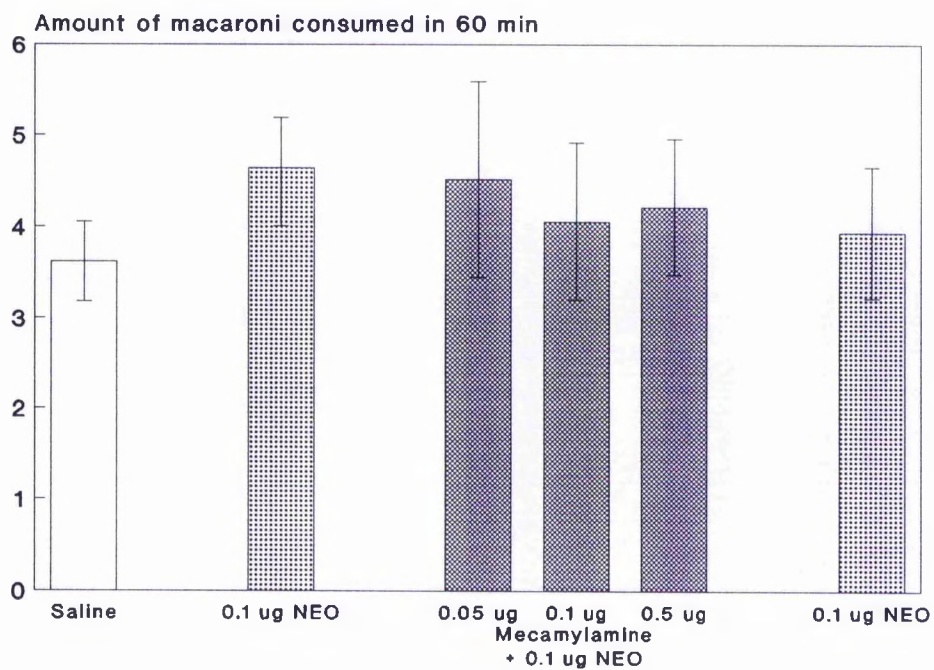


Figure 7:3

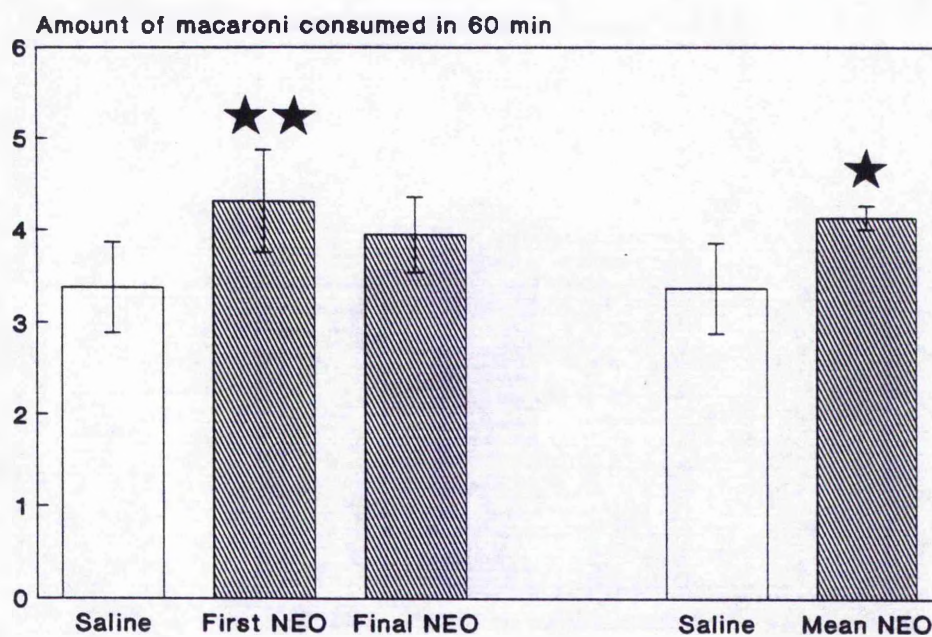
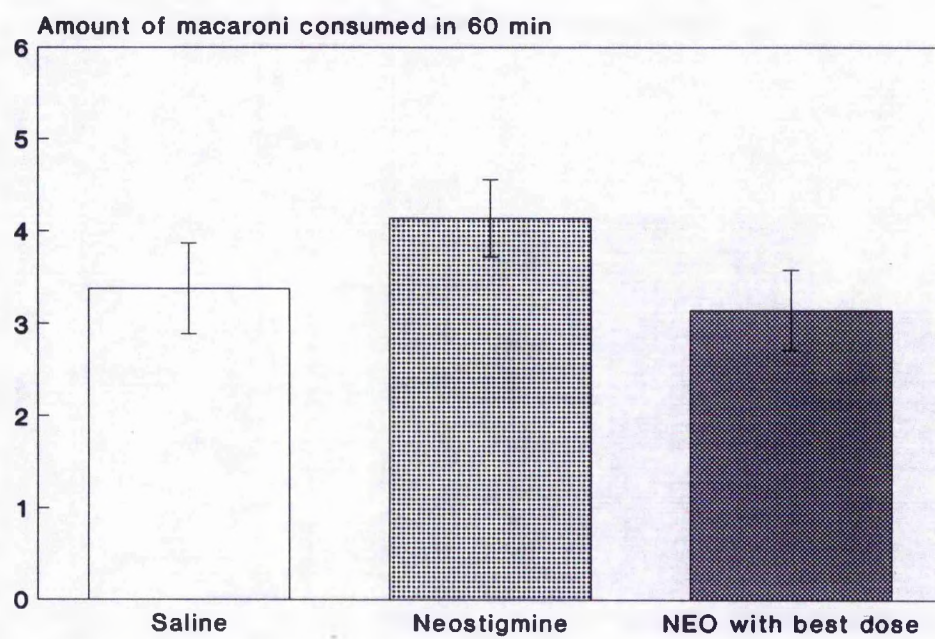


Figure 7:4



atropine or mecamlamine, although the effects of the antagonists were not discriminable and there was no clear effect of dose.

The demonstration that neither intranigral atropine nor mecamlamine decreased spontaneous feeding activity in satiated rats provides important information with respect to the type of innervation that the SNc receives. If the cholinergic projection to SNc was tonically active when the injection was made, then cholinergic receptor antagonists would be expected to induce a decrease in baseline feeding. As this does not occur, it implies that the cholinergic innervation of SNc was firing phasically during this period of testing. Although it has been shown that PPTg-Ch neurones have a tonic firing pattern (Kang and Kitai, 1990), subpopulations of cells in the PPTg-Ch are known to change their firing patterns during waking, slow-wave sleep and REM sleep (Garcia-Rill, 1991). It is therefore open to speculation whether a general property of PPTg neurones is that they can change their firing patterns according to the information they receive from synaptic inputs.

This study also extends several previous findings. First of all, in the previous study 0.1 μ g neostigmine was shown to be able to increase feeding of macaroni in satiated rats. In this study however, only 10 rats out of 20 which were tested showed this increased feeding response which suggests that responses following NEO may be as inconsistent as those following eserine. An explanation, which may explain the variable results of both eserine and neostigmine, relates to the proposed tonic nature of the cholinergic input to SNc at the time of the injection. Increases in behaviour following administration of AChEs depends upon the build-up of endogenous ACh in the synaptic cleft by blocking its hydrolysis. ACh build-up will depend upon the rate of firing of the neurone and if the presynaptic input fires phasically then this may occur in a rather indiscriminate fashion. Regardless of the reason for the variability, the responses obtained following NEO may be more

consistent in lower, tighter dose range: 0.1 μg was the lowest dose tested in the previous behavioural investigation and feeding activity decreased rapidly as the dose became larger, suggesting that 0.1 μg is one of the highest doses which can be used to stimulate feeding. The data from this study further indicate that for some rats even 0.1 μg is too high, implying that this dose lies at the boundary of effectiveness.

The second way in which this study extends previous data is in the types of antagonists used, the feeding activity investigated and the dose ranges tested. A previous study successfully blocked the increased feeding induced by a 5.0 μg ACh / 5.0 μg eserine injection mixture with 5.0 μg atropine (Winn *et al.*, 1983). The data from the first part of this study further demonstrate that antagonists injected into SN only attenuate the increased feeding response following cholinergic activation and not feeding behaviour *per se*. The data from the second part of the study demonstrate that the feeding response elicited following a 0.1 μg NEO injection can be attenuated by doses of 0.5 / 1.0 μg atropine or by doses of 0.1 / 0.5 μg mecamylamine. The fact that there were no observable differences between antagonist effects is not entirely surprising: it would be bizarre if the roles of muscarinic and nicotinic receptors normally co-excited on the same postsynaptic membrane were not complementary. However, the fact that the mean response following NEO with the "best dose" of antagonist totally attenuated the feeding response may be slightly more surprising. Why should there be total inhibition of the cholinergic effects by blocking one type of receptor when stimulation of each type individually produces feeding? (See effects following carbachol or nicotine in the previous chapter.) One possibility is that muscarinic and nicotinic receptors gate each other: both receptor-types may have to be available in order for activation of one by a direct agonist to be effective. Alternatively, ACh build-up following NEO injection may have weaker stimulatory power compared to specific stimulation of one receptor subtype by a direct agonist. A previous experiment

from this laboratory has indicated that the effects of muscarinic and nicotinic receptor activation are additive (Parker and Winn, 1992) and it may be that both are normally required for sufficient stimulatory intensity.

The magnitude of the feeding response following NEO was slightly decreased in the final injection as compared to the initial probe. This alteration might occur for one of two reasons. First, the effects of the antagonists may not be completely reversed over the 48 hr which were allocated between injections. Second, with multiple injections there is an increased probability of damage to neurones at the site of the injection which would decrease the efficacy of neuronal transmission there. The final injection of NEO was the sixth microinjection made at this site and histological examination of these rats suggested greater neuronal damage than was seen in rats given only 4 injections in the other part of the experiment.

The possibility that AChE inhibitors injected into the SN might affect transmission in the SNr has been suggested (Chapter 2). However the possibility that at least some of the increased feeding effects observed following NEO injection might be due to non-cholinergic mechanisms there has been refuted to some degree by these data. There were no residual effects on feeding behaviour attributable to NEO once the cholinergic receptors had been blocked, which suggests that if AChE does interact in some way with GABAergic transmission, its effects depend upon some prior cholinergic-mediated excitation at the receptors in the SNc. Such speculation makes sense in terms of the interactions which probably occur between the neurones of the SNc and SNr previously discussed in Chapter 2. The release of AChE from dendrites in the SNc may only occur when GABA transmission in the SNr is elevated (during periods of activity in specific motor output channels) and given the role of DA in the control of striatal output, GABAergic activity in the SNr would be controlled at least in part by cholinergic transmission in the SNc. By this route, specific inactivation of receptors in the SNc by atropine or

mecamylamine would also indirectly prevent release of AChE. Without its release there would be no additional sites at which NEO would be able to act in order to induce alternative behavioural output.

Conclusions

The data presented in this Chapter demonstrate that:

1. Intranigral injection of either mecamylamine or atropine does not affect spontaneous consumption of palatable food by satiated rats. This suggests that the cholinergic innervation of SNc is phasic rather than tonic.
2. The stimulatory effects of 0.1 μg NEO on macaroni consumption were variable across subjects, suggesting that this dose of NEO might be at the edge of the effective range.
3. Intranigral injection of either mecamylamine or atropine can attenuate the NEO-induced increase in consumption of palatable food by satiated rats, suggesting that stimulation of both receptors may normally be necessary.

8. Control of striatal dopamine by cholinergic neurones in the pons: a comparison of the acquisition of responding for conditioned reinforcement following amphetamine injections into the nucleus accumbens and neostigmine injections to the SN or VTA

Introduction

Previous investigations of the behaviours stimulated by cholinergic manipulation of SN have been straightforwardly descriptive and it has not been possible to determine what underlying behavioural mechanisms are affected. For instance, do the observed increases in feeding reflect changes in the rat's motivational state and if so, what sort of motivational changes does cholinergic stimulation of the SNc produce? Injections of cholinergic drugs into SN stimulate SNc DA neurones projecting to the dorsal striatum (Parker *et al.*, 1991; Blaha and Winn, 1993) and DA there is thought to be committed to the selection and initiation of appropriate motor responses. The exact nature of this selection is also dictated by the relative activity in corticostriatal projections and it seems additionally likely that selection of motor output at the level of the dorsal striatum can be influenced by ventral striatal motivational signals: part of the output from the core of the NAcc projects to the SNc (Heimer *et al.*, 1991).

It has been suggested that cholinergic stimulation of SN elicits dose-dependent increases in behaviours for which the animal has both a low current baseline rate of activity and a positive predisposition (Winn *et al.*, 1983). The low baseline rate of activity may be required so that an *increase* in DA release can be stimulated from the SNc - if the spontaneous firing of DA neurones has decreased, then stimulation in SN with cholinergic drugs might instigate a change in the existing behavioural pattern. If the "positive predisposition" is taken to imply a motivational inclination towards the stimulated activity then this again infers a direct influence in the control of behavioural expression by the ventral striatum. Whether such an

influence would be stimulatory (partially involved in initiating the activity) or modulatory (fine-tuning the specificity of the behaviour once it had been initiated) is unclear. This question may be evaluated by using an experimental paradigm which tests the acquisition of specific behavioural processes known to be dependent on the activity of mesolimbic DA neurones. A behavioural paradigm which can be used to assess a rat's partiality to responding for reward-related stimuli is that of acquisition of responding for conditioned reinforcement.

Different types of stimuli can act in distinct ways to affect behaviour. For example, they can elicit a response by acting as a discriminative stimulus or a reinforcer. A discriminative stimulus specifies that an action \rightarrow event relationship is in operation: for instance, for a hungry rat a tone can act as a discriminative stimulus to specify that pressing a lever before a set time period expires will deliver food. Presentation of a reinforcer increases the probability of repeating the responses which preceded it: for instance, if pressing a lever produces food for a hungry rat, then knowledge about this food delivery reinforces the lever-press and increases the probability of additional lever-press responses. A conditioned reinforcer is a specific form of stimulus-reward, where an initially non-reinforcing stimulus (such as a light or tone) acquires secondary reinforcing properties by being temporally paired with an unconditioned stimulus (such as food or water) (Mackintosh, 1974). Psychomotor stimulant drugs such as AMPH can enhance the rewarding effects of conditioned reinforcers, presumably by maintaining the strength of the connection with the unconditioned stimulus in its absence (Hill, 1970). Such drugs can propel the acquisition and sustain the output of a new operant response (a lever press) when it is reinforced only by the conditioned reinforcer (light / tone) in the absence of the unconditioned stimulus with which it has become associated (food / water) (Robbins, 1976; Robbins, 1978).

In recent years, interest has focussed on which aspects of the DA system mediate these associations. Taylor and Robbins (1984) showed that microinjection of AMPH directly into the NAcc enhanced responding for conditioned reinforcement (CR), while microinjection directly into the CPu produced inconsistent effects (see Chapter 3). Given the functional heterogeneity of the dorsal striatum, Kelley and Delfs (1991) more recently investigated the effects of AMPH injections into separate sub-regions of the striatum in the acquisition of responding for CR. Out of seven different striatal injection sites, only the NAcc and ventromedial caudate were found to be sensitive to the effects of AMPH in enhancing responding for CR and as the ventromedial caudate lies posterior to the NAcc in the same mediolateral and dorsoventral plane, this particular result might even be attributed to spread of injection fluid into the NAcc (the effects were observed only at the high dose, and were relatively small in magnitude). It may not be entirely surprising that the difficulty in eliciting consistent CR responding following CPu stimulation was unresolved by this method. As has been discussed, the dorsal striatum is functionally heterogeneous and receives a diverse afferent input from separate cortical regions (see Chapter 3). As such, the integration of cortical and DAergic inputs into one appropriate output message is likely to involve diverse combinations of stimulation which cannot be emulated by either a blanket flooding of the area with AMPH (Taylor and Robbins, 1984) or discrete AMPH infusions to dorsal striatal subcompartments (Kelley and Delfs, 1991). Therefore, inconsistent or negligible results by these methods cannot rule out the involvement of motivational factors in dorsal striatal outputs. Indeed, given the anatomical connection between NAcc core and SNc, it would seem unlikely that behaviours were selected without consideration of their motivational significance. Stimulation of DA neurones at the level of the SNc using the AChE inhibitor NEO is more likely to produce a recognised pattern of excitation across the CPu than either of the methods previously attempted. In fact in physiological terms, the potential integration of mesolimbic and nigrostriatal DA signals in motivationally significant

tasks (such as the acquisition of responding for CR) may be best investigated by this method.

Therefore in the present study direct comparisons were made between the roles of the dorsal and ventral striata in the acquisition of responding for CR by injecting NEO into the SN or ventral tegmental area (VTA). Given that central injections of AMPH are considered to produce exaggerated effects compared to DA itself (Cador *et al.*, 1991), the magnitude of expected responses following NEO injections were expected to be much smaller than following AMPH and it seemed appropriate to make a comparison between NEO injections into the SN and VTA. Many of the previous investigations of the CR paradigm have used a tone as the non-reinforcing stimulus which acquires reinforcing properties through a temporal pairing in the training phase with the primary reward, water (Robbins, 1976; Robbins, 1978; Robbins *et al.*, 1983; Taylor and Robbins, 1984; Taylor and Robbins, 1986). However the apparatus used in this laboratory paired a light with food similar to the procedure used by Kelley and Delfs (1991) and it seemed appropriate to ensure that this paradigm gave comparable results to those using tone / water. Therefore in this study data were collected from a separate group of rats with identical training following AMPH injections into the NAcc.

Methods

Animals

36 male Lister hooded rats with mean body weight at the time of surgery 265.17 (\pm 10.43 [SD])g. This was approximately 85% of the free-feeding body weight as rats were restricted in their food-intake for the duration of the experiment.

Surgery

Rats were anaesthetised with modified doses ($\sim 55 \text{ mg}\cdot\text{kg}^{-1}$) of sodium pentobarbitone and implanted bilaterally with stainless steel guide cannulae positioned above the NAcc, SN or VTA.

Intracranial microinjection

Rats were given injections of saline vehicle and drug (*d*-amphetamine sulphate: 10, 20, 30 μg ; neostigmine methyl sulphate 0.05, 0.1, 0.5 μg) in a random counterbalanced order.

Behavioural testing procedure

Rats were trained in the conditioned reinforcement paradigm before surgery and this training recommenced 7 days after surgery. Training continued post-operatively until each rat reached criterion once more (a minimum of 5 sessions), after which they were transferred to the testing phase.

Histological Analysis

Rats were sacrificed on completion of behavioural testing and microinjection sites verified in Nissl-stained sections.

Statistical Analysis

Total responses on each lever, total panel pushes and frequency of CRs were recorded at 3 min intervals during the 30 min test sessions. Lever-press and panel-press responses were analysed by parametric analysis of variance, with the data subjected to a square-root transformation to achieve homogeneity of variance as recommended by Winer (1971). Untransformed data were also analysed and results from each form of data analysis were similar.

Results

Histological analysis

Figure 8:1 presents representative sections indicating injection placements in the NAcc. Following histological examination, all ten rats from this group were found to have injection sites in the NAcc. Figure 8:2 illustrates representative sections indicating injection placements in the VTA and SN. Ten rats from each group were found to have injection sites in, or immediately adjacent, to the SN or VTA. Those rats found by histological inspection to have misplaced cannulae (3 rats from each group) were discarded from the behavioural analyses and are shown in Figure 8:2 as misplaced cannulae. These rats had higher rates of responding following saline than doses of NEO

Acquisition of responding for conditioned reinforcement

A 3-way analysis of variance was first carried out for lever- / panel-pressing at each dose and at each site to ascertain formally whether there were overall differences between the 3 groups. There were significant main effects of site ($F=5.67$ $df=2,27$ $p<0.01$) and what was being pressed ($F=50.99$ $df=2,54$ $p<0.001$), but not of dose ($F=2.20$ $df=3,81$). There were also significant interactions between site and what was being pressed ($F=5.71$ $df=4,54$ $p<0.001$) and site and dose ($F=3.97$ $df=6,81$ $p<0.005$), but there were no other interactions. As expected there was a huge difference in magnitude between responses following AMPH injections to NAcc and NEO to either the VTA or SN ($p<0.05$) and so AMPH and NEO data were re-analysed separately.

Responding for CR following intra-accumbens AMPH. Figure 8:3 illustrates the mean response rates on each lever (CR / NCR) and the food hopper panel following microinjections of saline and each dose of AMPH. Responding on the separate levers and the panel was significantly different ($F=31.11$ $df=2,18$ $p<0.001$) and there was also a main effect of dose of AMPH ($F=7.53$ $df=3,27$ $p<0.001$) but

Figure 8:1

Representative sections, redrawn from the atlas of Paxinos and Watson (1982) showing placements for nucleus accumbens injection cannulae (filled circles).

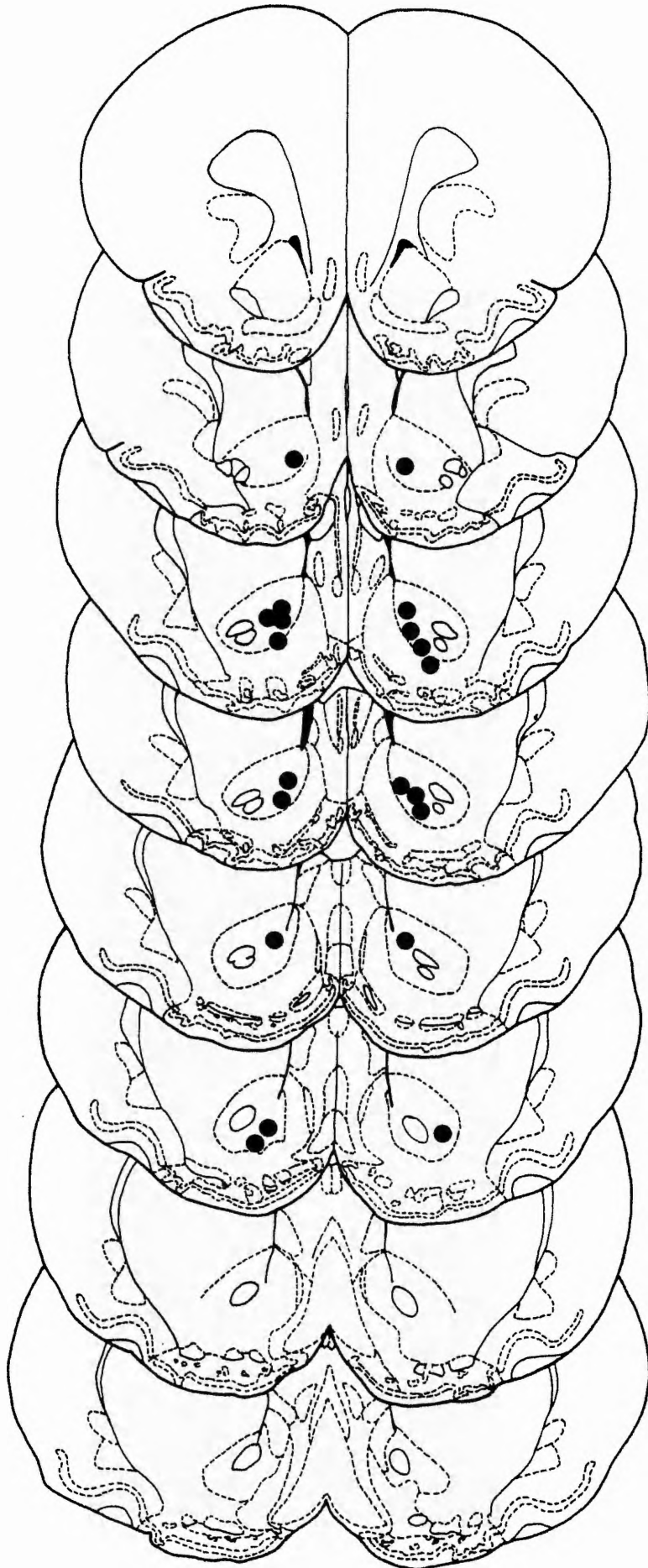
Figure 8:2

Representative sections, redrawn from the atlas of Paxinos and Watson (1982) showing placements for substantia nigra (SN) and ventral tegmental area (VTA) injection cannulae (filled circles). Misplaced injection sites are also illustrated (filled triangles).

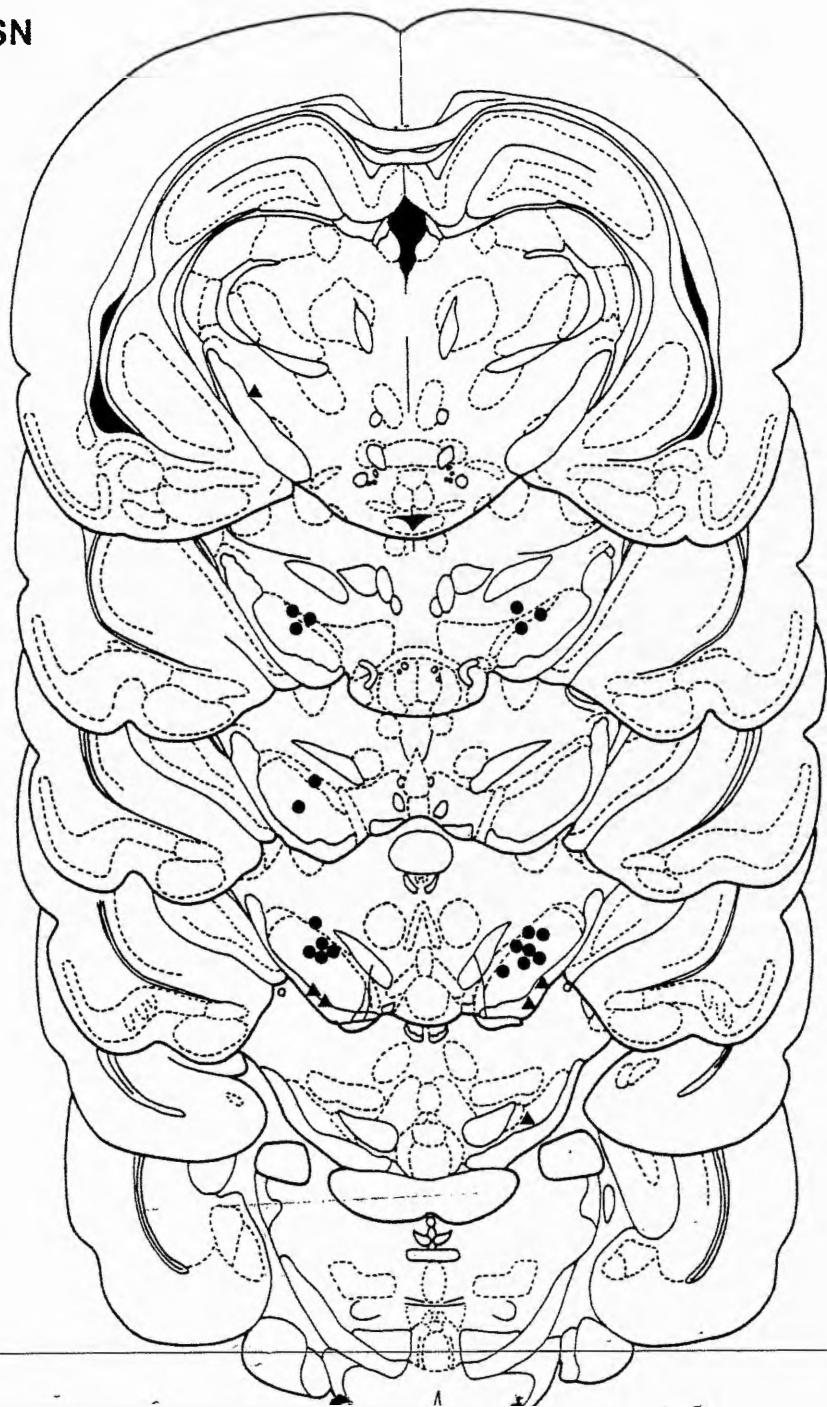
Figure 8:3

Dose-effects of bilateral microinjection of AMPH to the nucleus accumbens on responding on a lever providing conditioned reinforcement (CR), a lever providing no conditioned reinforcement (NCR) and a panel which gave access to the primary reward in the training phase of the experiment. Responding on the CR lever and panel was significantly higher than on the NCR lever and responding following 20 μg and 30 μg AMPH was significantly different to saline.

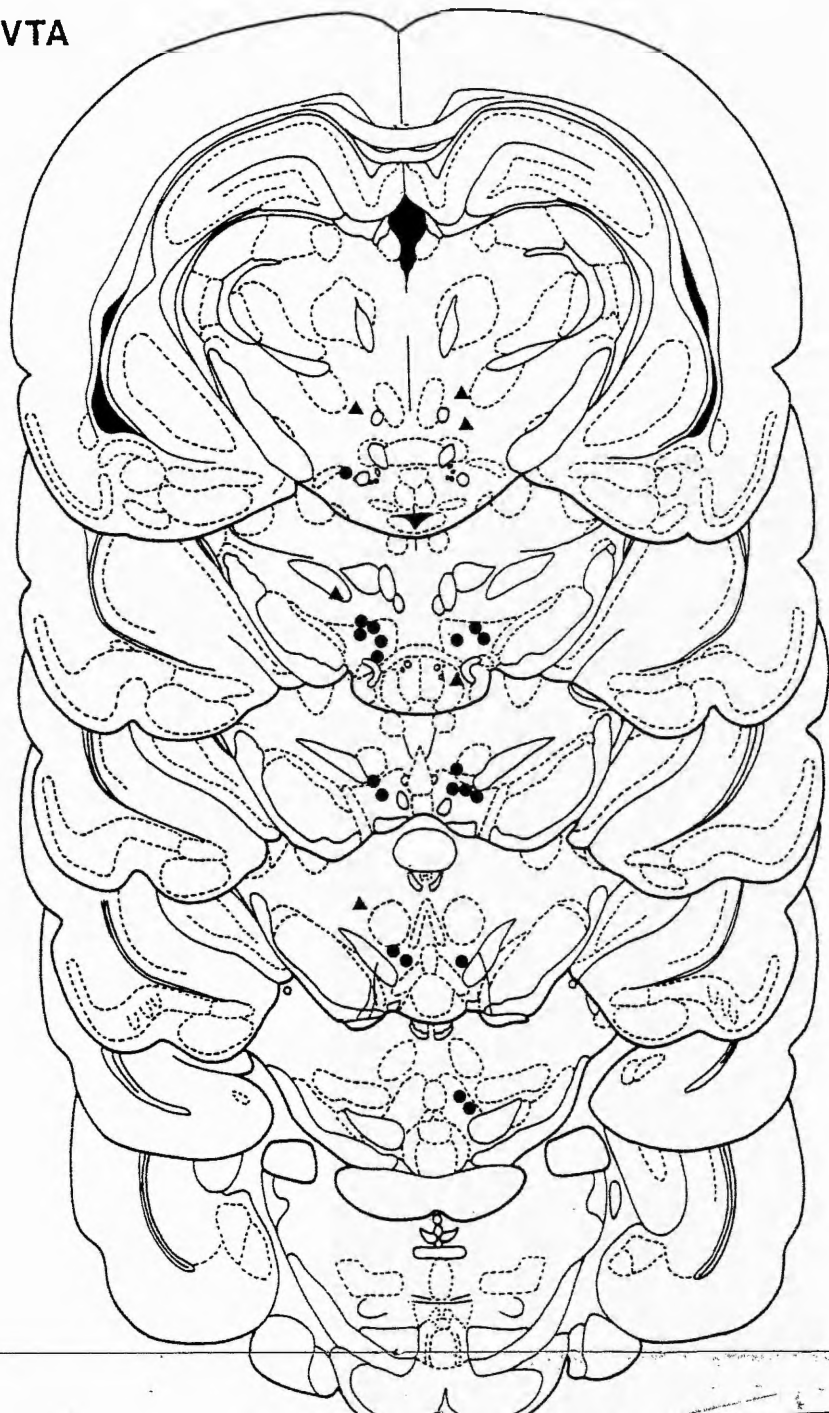
NAcc



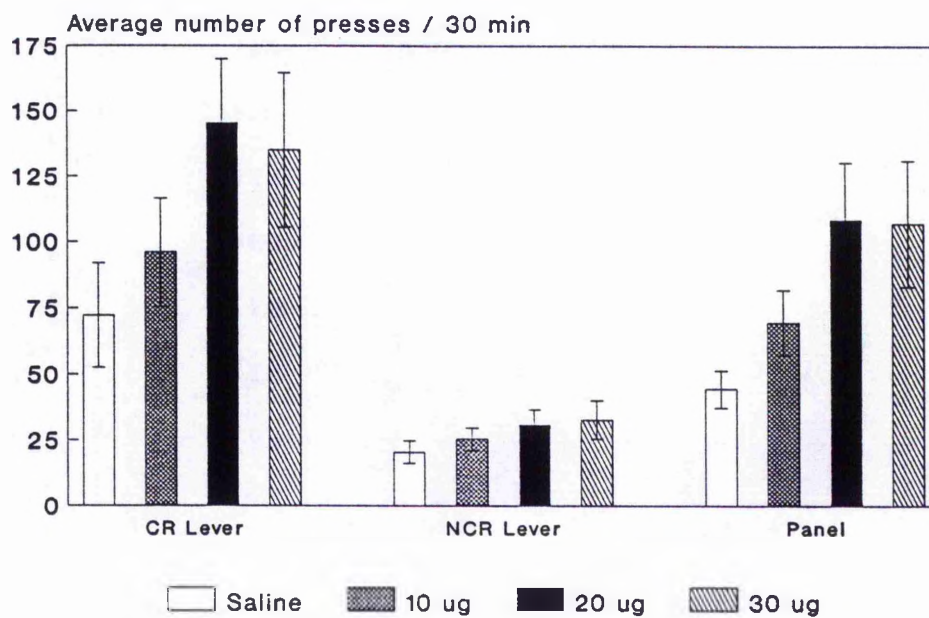
SN



VTA



Nucleus Accumbens



no interaction ($F=1.42$ $df=6,54$). *Post hoc* tests revealed that rats responded significantly more on the CR lever and panel than on the NCR lever (both $p<0.001$) and that responses were higher following infusions of 20 μ g and 30 μ g AMPH than following saline ($p<0.005$ and $p<0.01$ respectively).

Responding for CR following intra-SN or intra-VTA neostigmine. Figure 8:4 illustrates the mean response rates on each lever and the food hopper panel following microinjections of saline and each dose of NEO to the VTA or SN. There were no significant main effects of site ($F=0.00$ $df=1,18$) or dose ($F=1.90$ $df=3,54$) but there was an effect of what was being pressed (CR lever, NCR lever or food hopper panel) ($F=22.61$ $df=2,36$ $p<0.001$). There were no significant interactions. *Post hoc* testing revealed that both groups of rats responded less on the NCR lever than on either the CR lever or the panel (both $p<0.001$).

In a previous study (Parker and Winn, submitted 1993), there were also no differences between injection sites (VTA vs. SN) in the acquisition of responding for CR following injections of a carbachol/nicotine mixture. However, in that study, dose-dependent increases in responding for CR were shown to be dependent upon the baseline rate of panel pressing. Therefore, a similar rate-dependency analysis was conducted for the present data. First, a Pearson correlation coefficient was calculated by comparing panel pressing in response to saline with the change in panel pressing following NEO (calculated as the mean of the responses at each dose minus the response following saline). There was a correlation of $r=-0.70$, with a statistical significance of $p<0.001$ (Figure 8:5). Second, given that the change in panel pressing following NEO was significantly correlated to the response following saline, the rats were divided into low and high baseline panel-pressing groups by means of a median split. In this study neither individual baseline group showed a dose-dependent increase in panel pressing (Figure 8:6) (low: $F=2.18$ $df=3,27$; high: $F=2.12$ $df=3,27$) and there were no significant interactions of

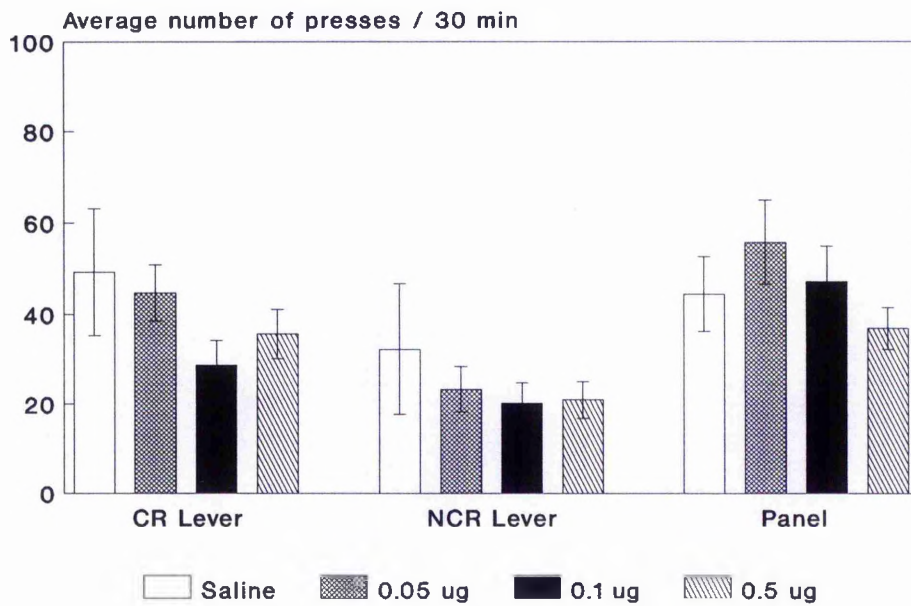
Figure 8:4

Dose-effects of bilateral microinjection of NEO to the ventral tegmental area or substantia nigra on responding on a lever providing conditioned reinforcement (CR), a lever providing no conditioned reinforcement (NCR) and a panel which gave access to the primary reward in the training phase of the experiment. Responding on the CR lever and panel was significantly higher than on the NCR lever but there was no effect of dose or site.

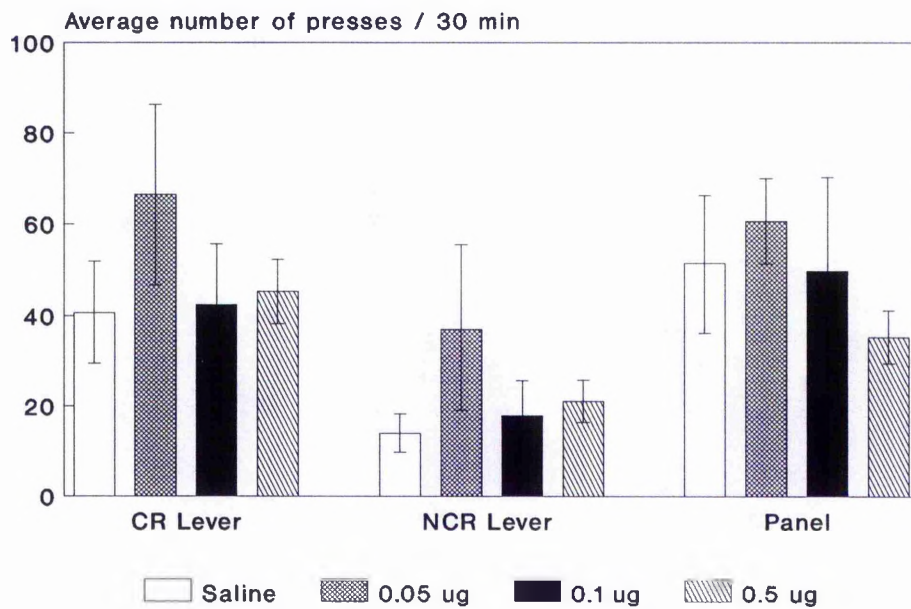
Figure 8:5

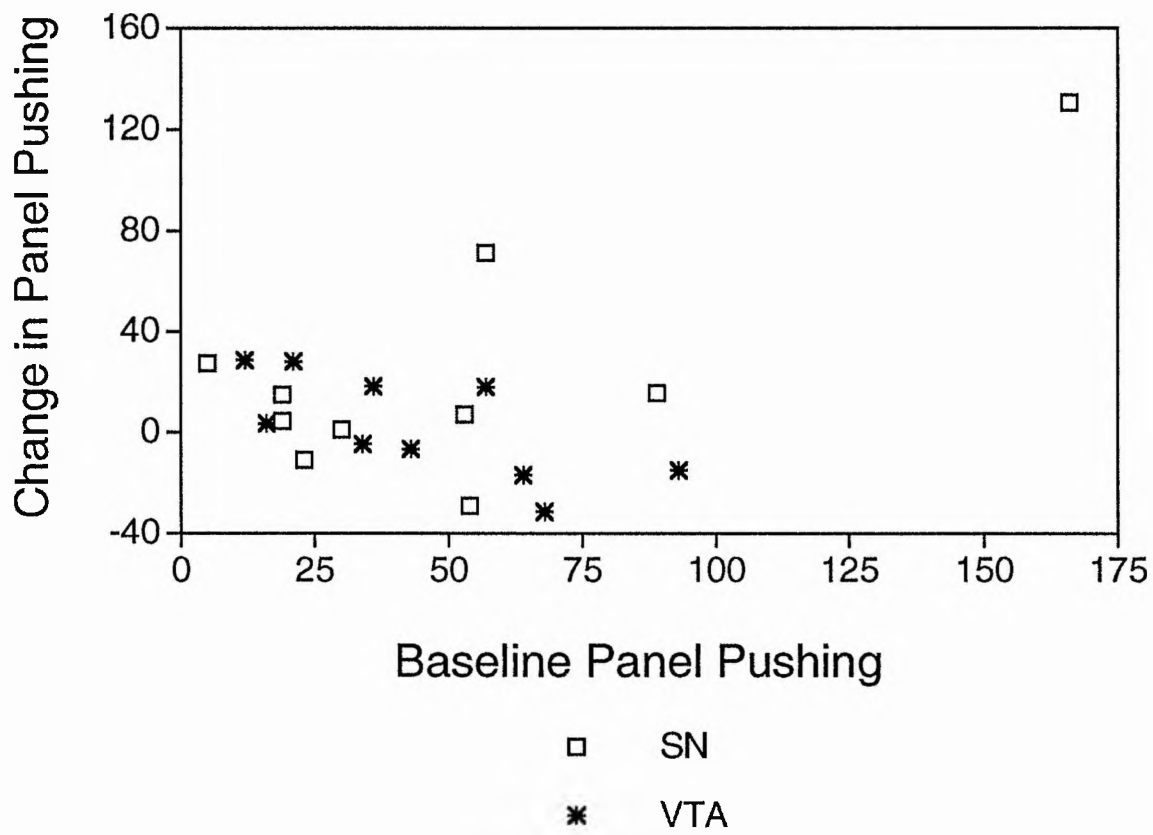
Scatterplot illustrating the significant correlation ($r=-0.70$ $p<0.001$) between baseline (saline) panel pressing and the change in panel pressing following cholinergic stimulation of SN or VTA (the mean response following neostigmine minus the saline response).

Ventral tegmental area



Substantia nigra





baseline group with injection site (group \times site: $F=2.35$ $df=1,16$; group \times site \times dose: $F=0.40$ $df=3,48$). This result differs from the one obtained in the previous study using carbachol/nicotine mixtures (Parker and Winn, submitted 1993), where significant dose-dependent increases in panel-pressing were obtained in the low, but not high, baseline group. This difference might be explained by the less variable response set (extinguishing inappropriate panel-pressing) which the rats in the present study achieved through more extensive training.

Similarly, in the previous study (Parker and Winn, submitted 1993), there was a significant dose-dependent increase in CR lever-pressing in the low baseline group and a significant dose-dependent decrease in CR lever-pressing in the high baseline group. The lever-pressing data in the present study were re-analysed (Figures 8:7 and 8:8) with main effects of site (SNc or VTA), baseline panel-pressing (low or high), lever-pressing (CR or NCR) and dose (saline and 3 doses of NEO). There were no main effects of site ($F=0.13$ $df=1,16$), baseline panel-pressing group ($F=3.31$ $df=1,16$), or dose ($F=2.08$ $df=3,48$), although there was a significant main effect of lever as was expected from the initial analysis ($F=46.37$ $df=1,16$ $p<0.001$). There was also a site by baseline group by lever interaction ($F=5.96$ $df=1,16$ $p<0.05$): the predominant result from *post hoc* tests was that the high baseline panel-pressing SN-infused rats pressed significantly more on the CR lever than did any other sub-group of rats (high or low baseline, SN or VTA) on either lever (all $p<0.01$). A separate analysis of lever-pressing showed that the main effect of dose approached significance for the CR lever ($F=2.74$ $df=3,48$ $p=0.053$) but not the NCR lever ($F=1.00$ $df=3,48$) and that there was a significant site by baseline group by dose interaction for the CR lever ($F=3.28$ $df=3,48$ $p<0.05$) but not the NCR lever ($F=0.88$ $df=3,48$). No other main effects or interactions were significant. *Post hoc* tests revealed that 0.05 μ g NEO injected into SN in the high baseline group stimulated significantly higher rates of CR lever-pressing than all

Figure 8:6

Panel-pressing in the high and low baseline panel-pressing groups (designated by a median split) at each site.

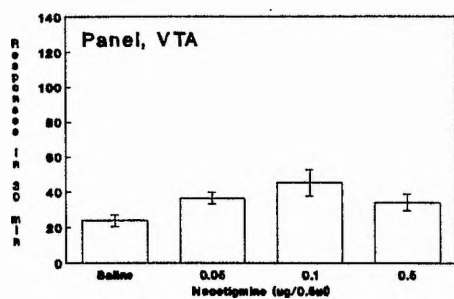
Figure 8:7

CR lever-pressing in the high and low baseline panel-pressing groups.

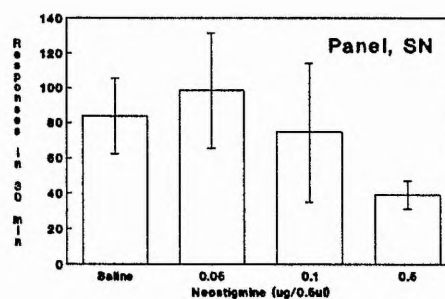
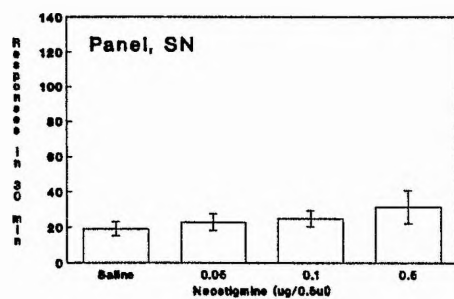
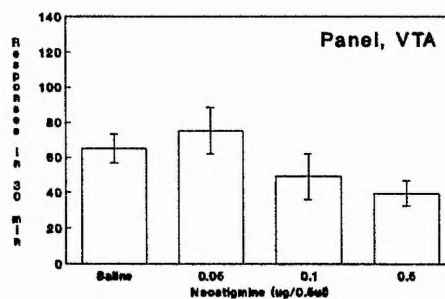
Figure 8:8

NCR lever-pressing in the high and low baseline panel-pressing groups.

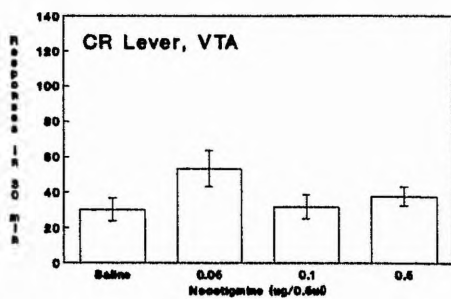
LOW BASELINE PANEL PRESSING



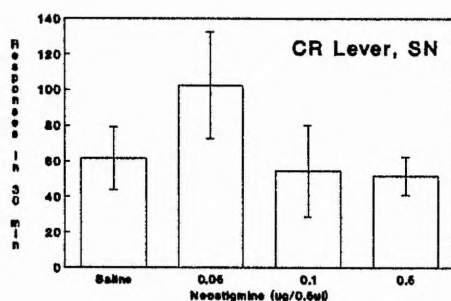
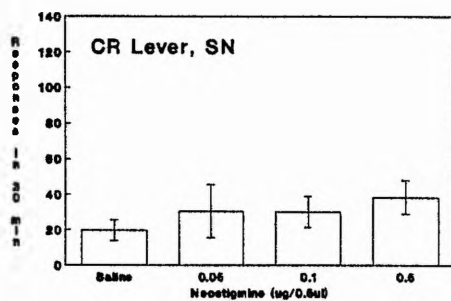
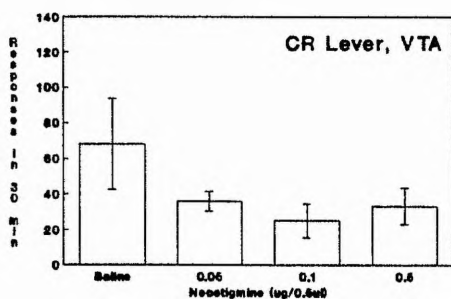
HIGH BASELINE PANEL PRESSING



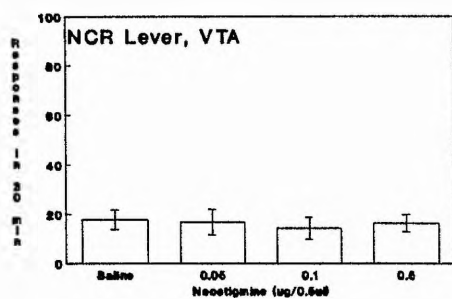
LOW BASELINE PANEL PRESSING



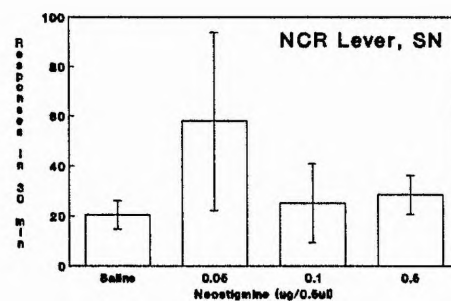
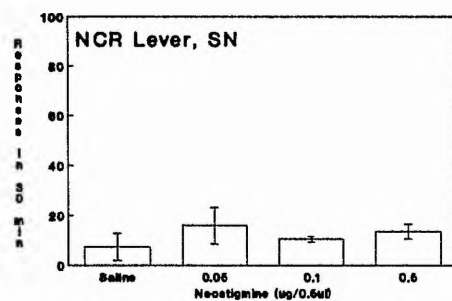
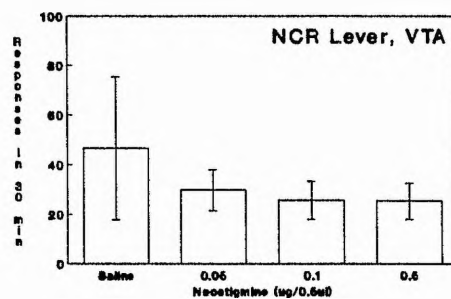
HIGH BASELINE PANEL PRESSING



LOW BASELINE PANEL PRESSING



HIGH BASELINE PANEL PRESSING



SN doses in the low baseline group, all VTA doses in the high baseline group and all doses except 0.05 μg in the VTA low baseline group (all $p < 0.01$).

Given the inconsistent results obtained following injections of anticholinesterases to SN (see previous 2 experiments), a "best dose" analysis of these data was also carried out. The dose of NEO at which the greatest CR lever-pressing was obtained was designated the "best dose" for that rat. It can be seen from Figure 8:9 that the mean best dose of NEO in the SN group is much clearer to identify from higher and lower doses than in the VTA group. There was a significant main effect of best dose of NEO compared to saline ($F=5.63$ $df=1,18$ $p < 0.05$) and although there was no significant main effect of site ($F=0.38$ $df=1,18$) there was a significant interaction ($F=5.63$ $df=1,18$ $p < 0.05$). *Post hoc* tests showed that responding on the CR lever following the best dose of NEO in SN-injected rats was significantly increased compared to saline ($p < 0.05$) while responding on the CR lever following the best dose of NEO in the VTA group was not different to saline ($p=1.00$) (Figure 8:10). Comparing the corresponding scores for NCR lever- and panel-pressing with their saline data (Figure 8:10) demonstrated no significant differences between sites (NCR: $F=0.03$ $df=1,18$; Panel: $F=0.53$ $df=1,18$) or saline and drug (NCR: $F=0.65$ $df=1,18$; Panel: $F=1.28$ $df=1,18$) and no significant interactions (NCR: $F=2.81$ $df=1,18$; Panel: $F=0.17$ $df=1,18$).

Discussion

Microinjections of AMPH into the NAcc or NEO into the SN or VTA all supported the acquisition of responding for CR (as determined by the greater pressing recorded on the CR than the NCR lever in all groups). The magnitude of responding was much greater following AMPH than NEO. A dose-dependent increase in responding on both the CR lever and the panel were found following AMPH. There was no superficial difference between responses following NEO injections to the SN or VTA and there were no dose-dependent effects of the drug

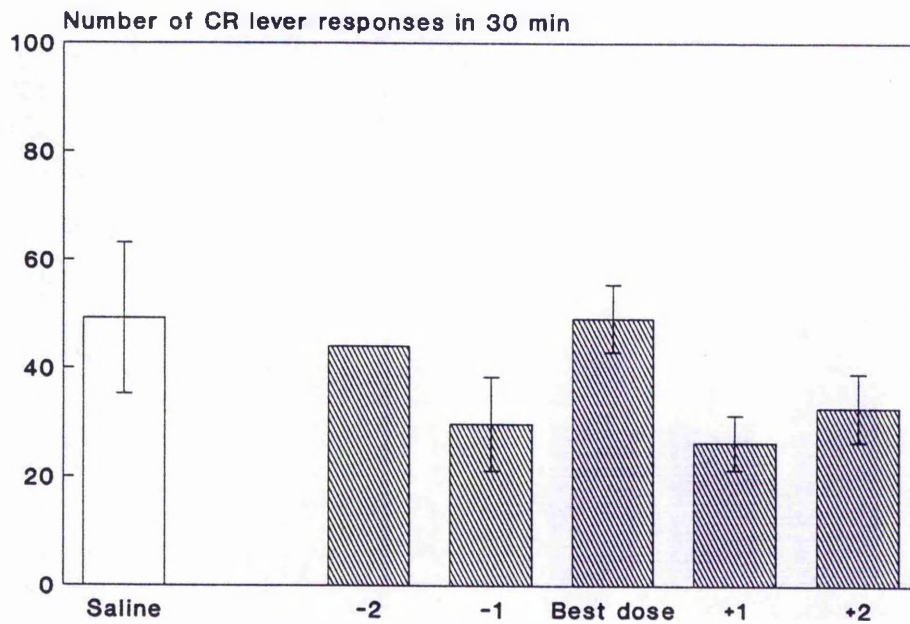
Figure 8:9

Mean number of CR responses in 30 min following injections of the "best dose" of neostigmine. The means from the corresponding higher / lower doses were also calculated and plotted: the mean best dose response is more clearly different to other responses in the SN than the VTA.

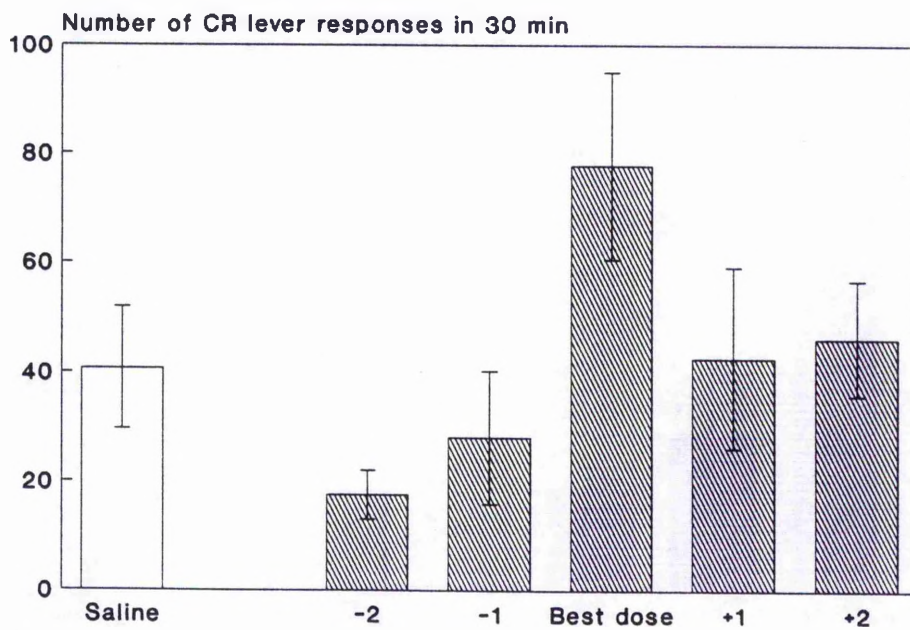
Figure 8:10

The best dose response on the CR lever in 30 min and the corresponding best dose response on the NCR lever and panel compared to saline at each site. The best dose of neostigmine induced significantly greater responding than saline on the CR lever following injection to the SN ($p < 0.05$).

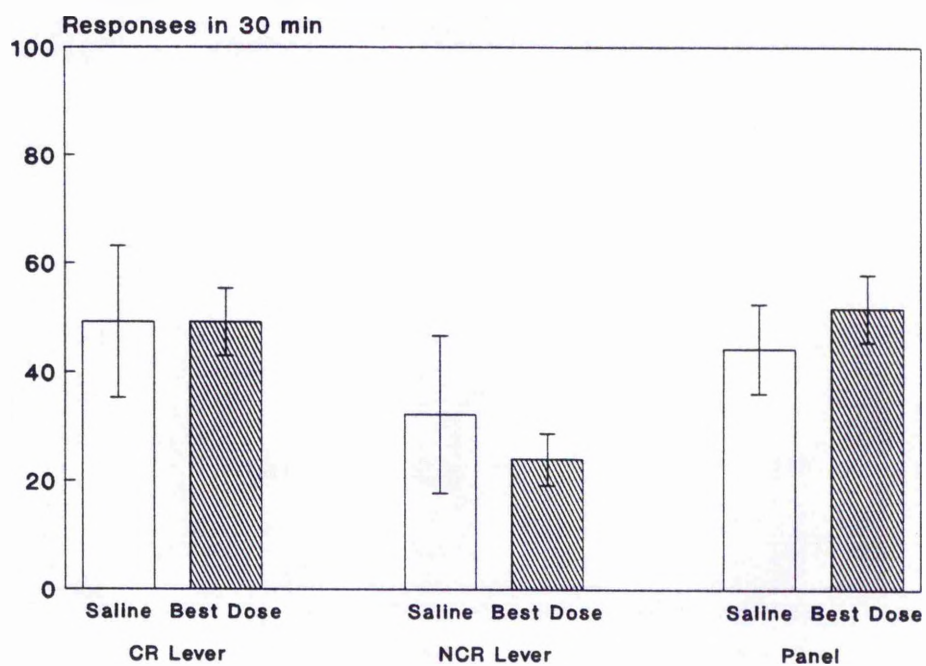
Ventral tegmental area



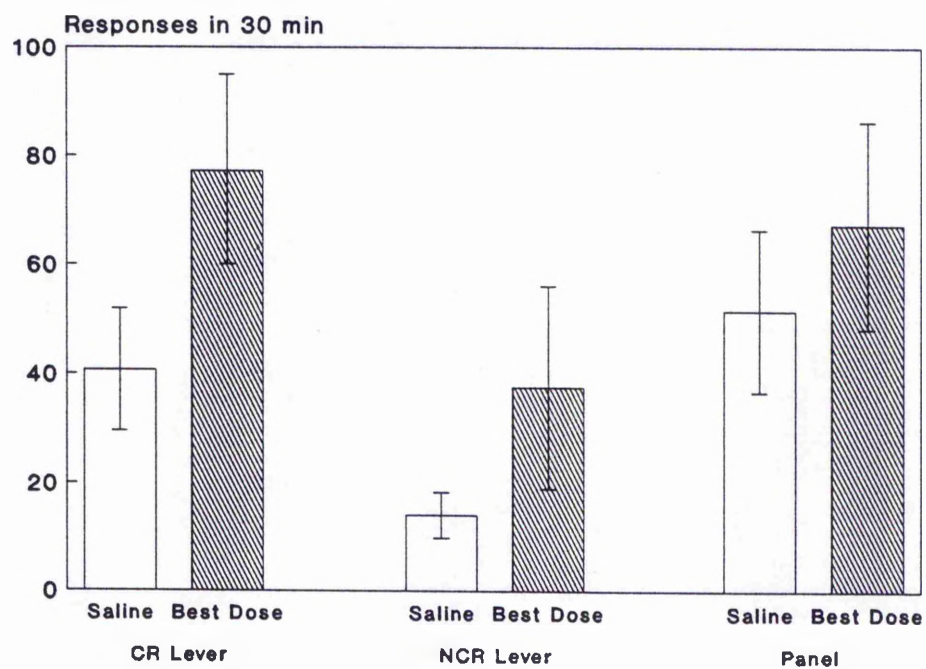
Substantia nigra



Ventral Tegmental Area



Substantia nigra



overall or in high and low baseline panel-pressing groups following a median-split. A best dose analysis revealed a somewhat surprising increase in responding on the CR lever (but not NCR lever or panel) in the nigral, but not VTA rats.

Responding for CR following NAcc AMPH

The present results confirm the previous findings that infusion of AMPH into the NAcc selectively stimulates responding for CR (Taylor and Robbins, 1984; Kelley and Delfs, 1991). More specifically they confirm the results obtained by Kelley and Delfs, who also used food rather than water as the primary reward, indicating that the development and enhancement of CR responding is independent of the type of primary reward used for conditioning. However, it should be pointed out that the magnitude of the results obtained following a food pairing were considerably smaller than those obtained following a water pairing. For instance at a dose of 20 μ g using food, Delfs and Kelley obtained a mean of approximately 125 CR responses in 30 min which compares well with the present study (~140 CR responses/30 min), while using water at the same dose, Taylor and Robbins obtained a mean of approximately 300 CR responses over the same period of time. Delfs and Kelley attributed this difference to the injection volumes (Delfs and Kelley: 0.5 μ l; Taylor and Robbins: 2 μ l) but as the injection volume for the NAcc was 2 μ l in this study and responses were again smaller than in the Taylor and Robbins (1984) study, it seems more likely that they were due to the difference in primary reward used. Water is far more indispensable to an animal in the short term than food and therefore a rat which has been deprived of water overnight is likely to learn a stronger association between the primary reward and conditioned reinforcer than a rat which has been deprived of *ad lib* access to food. However, while this effect is interesting, the important point for the purposes of this study is that with the paradigm which was used here, with food as the primary reward, the acquisition and continuation of responding for CR occurred.

The difference in magnitude of responding following NAcc AMPH and SN / VTA NEO is also an important issue. It is pertinent to discuss this in the context of differences in the magnitude of response achieved following AMPH or DA microinjections to the NAcc. Intra-NAcc DA administration led to much weaker increases in responding than AMPH infusions (20 μ g DA / 2 μ l gave a mean of approximately 80 CR responses in 30 min) (Cador *et al.*, 1991). This difference demonstrates that the low rates of responding obtained following NEO are not inconsistent with the size of the effects of DA injected directly into the striatum. Rather, it is the mechanism by which AMPH has its DAergic effects which stimulates abnormally high response rates. As discussed in Chapter 6, AMPH blocks re-uptake of DA from the synaptic cleft and releases all freshly synthesized DA from the presynaptic cytoplasm (Butcher *et al.*, 1988). Therefore, the volume of DA which can be released by AMPH is far in excess of that which could be released from the neurone in normal circumstances, and this therefore explains the exceptionally high CR response rates obtained in this and other studies.

Responding for CR following SNc or VTA NEO

An entirely superficial view of these data suggests that there is no difference in responding for reward between cholinergic stimulation of VTA and SN. This is somewhat surprising as the traditional viewpoint links the VTA and NAcc together with "reward"-related tasks while the SN and CPu are considered rather differently to be "motor"-related. A second interpretation of the data, using the best doses of NEO, produced an even more unexpected result, with the drug-dependent effects on the CR lever being restricted to the SN rats, with no effect compared to saline in the VTA group. This surprising result must be viewed alongside apparently contradictory data from other studies which measured the acquisition of reward-related behaviours through cholinergic stimulation of midbrain DA neurones. A previous study, which has already been referred to, investigated CR acquisition and found there to be no difference between responses following carbachol and nicotine

injections to the SN or VTA (Parker and Winn, submitted 1993). Furthermore, in a conditioned place preference paradigm rats formed a weak but positive place preference to nicotine when it was injected into the VTA, but not the SN (Winn *et al.*, unpublished observations).

These data may not be as paradoxical as they first appear. In fact the disparate results may be related to the degree of repetition (habit) associated with performance of the task. In a relatively novel test environment such as the conditioned place preference paradigm, the rat must make an association with reward and use this to shape its behavioural output. On the other hand in the operant chamber in the present study, the rat has already made a strong association with reward during the extensive training procedure and must transfer this learned motivational significance to a novel action (lever-pressing).

Ljungberg and colleagues (1992) demonstrated that in a novel task DA neurones would fire in response to orientation towards a novel stimulus, presentation of a primary reward as a reinforcer during conditioning and presentation of a conditioned stimulus which has become associated with the presentation of the primary reward. However with continued training, neuronal responses decreased both in terms of numbers of neurones responding and magnitude of responses, suggesting decrement of attentional and incentive processes as responding to the cues became habit. This drop in firing is actually quite logical in terms of the presumed role of DA in the striatum: in a novel task there will be great neuronal bombardment of the striatum from separate cortical regions all carrying their own important information about the situation in which the animal finds itself. The role of DA in promoting response selection is very important at this stage as it must help to highlight certain inputs and minimize others to produce a decisive first response. This first response will then be recurrently modulated both with the help of feedback from modified cortical signals and phasic firing of DA neurones. As the

objective becomes clear, a response set can be formed with the assistance of DAergic mechanisms (Robbins and Brown, 1990). Performance of the response set gradually becomes more routine and less attention is given to unimportant factors in the environment, corticostriatal firing is likely to become specific to important cues and DA neurones are gradually likely to cease firing.

Learning a new response (for instance a lever press) in a familiar environment where the levers are considered irrelevant will require reinstatement of A9 DAergic influences to alter the existing behavioural response set. It has been argued from animal learning theory that if an animal learns that 2 events are unrelated (for instance in this task during training, lever-pressing and light / food presentation are unconnected) it is harder to learn about any subsequent association between them (Mackintosh, 1973). This suggests that reinstatement of DAergic mechanisms to "over-write" an existing learned behavioural repertoire is a substantial requirement. The automatic first response of a rat placed in an operant chamber for the *n*th time is to check if there is food in the hopper and if not, to wait; the levers do not form a part of the existing response set. Therefore clear acquisition of responding for CR will not normally occur in a very well-trained rat unless sufficient supplementary stimulation of the A9 DA system occurs.

The rats tested by Taylor and Robbins (1984) had 14 consecutive days of training compared to 24 days in the Parker and Winn (1993) study and between 20 and 50 days (depending on individual performance) in the present study. Therefore the expectation of reward in each session was most firmly established in the individuals in the present study and least firmly in those in the Taylor and Robbins study. AMPH injections into the NAcc (Taylor and Robbins, 1984) would have stimulated mesolimbic DA to enhance the motivational importance of the task and the NAcc would additionally have sent increased output to the SNc to stimulate nigrostriatal DA. As these rats were not over-trained, the A9 neurones would be

active in any case to help define an appropriate response and the NAcc input would serve to establish a response based on the reward-related nature of the task. Even with increased length of training in the present study, it appears that AMPH has sufficient DA-releasing power in its indirect stimulation of the SNc to overcome the effects of routine. AMPH injections to the CPu on the other hand would simply release additional DA there without particular reward-related guidance of motor output: the actual responses observed would be more likely to depend upon individual perception of reward and this may account for the inconsistencies in CR responding observed following CPu injections (Taylor and Robbins, 1984).

The smaller magnitude of responses following NEO may be more susceptible to the effects of habit formation. NEO injections into the VTA will stimulate DA release in the NAcc although to a lesser extent than AMPH. Well-trained rats expect reward to be produced in a specific way and will therefore require substantial stimulation in CPu to overcome familiarity. The indirect connection from NAcc to SNc in this case is unlikely to be sufficient for this purpose and as such the rats did not respond more on the CR lever following NEO compared to saline. Conversely, NEO injections to the SN would increase firing of dorsal striatal DA neurones to provide ideal conditions for modification of the response set. Well-trained rats would expect reward in the operant chamber and the increased DA in the CPu would allow alteration of motor output to transfer the perceived rewarding nature of the chamber to a new motor act. It is particularly interesting in this regard that the increase in CR lever-pressing in nigral rats was largest in the high baseline panel-pressing group, as these were the rats which were most actively seeking reinforcement.

In the case of conditioned place preference, the relative novelty of the situation ought to lead spontaneously to increased A9 DA firing in order to form a response set. Therefore in this case NAcc AMPH and VTA NEO would be expected to

produce similar results (although possibly different in magnitude) as experimental stimulation of mesolimbic DA associated the situation with reward and integrated this in the formation of an output response. Conversely injections of nicotine into SN would increase DA release in an already actively firing A9 pathway without the added influence from mesolimbic DA to stimulate the formation of a conditioned response. Therefore, in this more novel situation VTA, but not SN, injections induced formation of a conditioned place preference.

If the response observed following cholinergic manipulation of VTA or SN is indeed contingent upon the degree of habit involved in the task performance, then there must be a crossover point where there would be no difference between the behaviours observed following stimulation of either SN or VTA. Indeed this was what was found in the other CR study discussed (Parker and Winn, submitted 1993) where the rats were not as well-trained as those in the present study.

Conclusions

The data presented in this Chapter demonstrate that:

1. Acquisition of responding for CR where the primary reward was food can be supported by microinjections of AMPH into the NAcc. Responding on the CR lever and food-hopper panel is dose-dependent.
2. Acquisition of responding for CR can be supported by microinjections of NEO into the SN, but in this case not the VTA. Together with data previously collected from this laboratory, these suggest that the effects of cholinergic stimulation of midbrain DA neurones may depend on the degree of habit associated with task performance.

9. Outflow from the striatum: effects of PPTg lesions on spontaneous locomotion, drug-induced locomotion and stereotypies

Introduction

There is clear anatomical evidence to suggest that the PPTg-nCh is an output station for the striatum (see Chapter 4). However the most significant practical problem in dealing with the PPTg has been the lack of any well-established lesion technique and this problem could easily account for many of the discrepancies in the literature previously described. For instance, the claim that the PPTg mediates NAcc-stimulated locomotion is based principally on data obtained by stimulating or inactivating the PPTg in acute procedures (Brudzynski and Mogenson, 1985; Milner and Mogenson, 1988; Mogenson and Wu, 1988; Garcia-Rill *et al.*, 1990; see Chapter 4). Lesions of the PPTg using a variety of excitotoxins resulting in inconsistent amounts of damage have occasionally affected locomotor activity (Brudzynski and Mogenson, 1985; Bechara and van der Kooy, 1992c) but more often have not (Swerdlow and Koob, 1987; Dellu *et al.*, 1991; Olmstead and Franklin, 1992) (see Chapter 4).

We have made a considerable effort to formulate an acceptable lesion procedure for this structure. This has involved comparing the actions of various excitotoxins to examine their effect on cholinergic and non-cholinergic neurones (Rugg *et al.*, 1992) and to define co-ordinates: the shape of the PPTg demands that more than one lesion placement is used in order to make effective lesions (Dunbar *et al.*, 1992). The present study was undertaken to determine whether or not lesions of the PPTg using this method have any effect on behaviours known to be mediated by the CPu or NAcc. Lesions were made by ibotenate, a toxin able to produce damage predominantly restricted to the PPTg and destroying virtually all neurones within it (Rugg *et al.*, 1992). Stimulation of the CPu or NAcc was achieved using systemic administration of *d*-amphetamine or apomorphine. The effects of lower

doses of these drugs on locomotion is known to be mediated by the NAcc and the effects of higher doses on stereotyped behaviour by the CPu (Kelly *et al.*, 1975; Kelly and Iversen, 1976). In addition, ibotenate lesions were made in the deep mesencephalic nucleus (DpMe), adjacent to the PPTg, in order to discriminate between different lesion placements in this complex pontine region. The DpMe is likely to include a heterogeneous mixture of neurones, and has connections with a variety of structures. It may be an important output station for the zona incerta (ZI), a subthalamic structure that has both somatosensory and somatomotor functions (Shammah-Lagnado *et al.*, 1985; Nicolelis *et al.*, 1992). The DpMe has also been linked to motor outflow (Brotchie *et al.*, 1991). The present study attempted to discover whether lesions in these regions were dissociable both in terms of histology and basic motor behaviours.

Materials and Methods

Animals

25 male Lister hooded rats (Harlan Olac) with mean body weight $342.4 (\pm 19.1$ [SD])g at the time of surgery.

Surgery

Rats were divided into the following groups: PPTg ibotenate ($n = 7$), DpMe ibotenate ($n = 8$), PPTg phosphate buffer control ($n = 5$) and DpMe phosphate buffer control ($n = 5$). All rats were anaesthetised with sodium pentobarbitone. Despite staggering surgery such that bilateral lesions were made over 2 consecutive days, 2 rats in the PPTg ibotenate group died within 24 h of the second day of surgery, reducing the number of this group to 5. All their pre-operative data were discarded.

Body Weight, Spillage, Food and Water Intake

Body weight, food and water intake and food spillage (collected on foil trays) were recorded for each rat for 7 days prior to surgery and for 14 days after to determine whether or not the lesions induced any gross disturbances in regulatory mechanisms and also to monitor post-operative recovery. Once food and water intake had returned to pre-operative levels and remained stable for several days locomotor testing began.

Drug-induced locomotion and stereotypy

Locomotor testing began on the 15th post-operative day and continued for 21 consecutive days. Rats were placed in 12 wire locomotor cages (0.38 m x 0.24 m x 0.19 m) through which passed 2 infra-red light beams. Each time a beam was interrupted a count was registered by a microprocessor ("Spider" Paul Fray Ltd.). The beams had to be broken sequentially, to avoid misrepresenting as locomotion other behaviour directed towards one beam only, such as sniffing. The position of lesion groups was balanced across the rack of cages to minimize the possibilities of cage sensitivity affecting the scores. Each session lasted 1 h and took place under red light illumination; rats were continuously observed in double-blind trials.

After 21 days' measurement of locomotion in the undrugged state, pharmacological studies began. Locomotion and stereotypy were examined following systemic injection of the DA stimulants *d*-amphetamine sulphate (AMPH) and apomorphine hydrochloride (APO) (Sigma Chemicals). Three different doses of AMPH (1.5, 3.0 and 5.0 mg·kg⁻¹ dissolved in 0.9% saline) were administered on alternate days; saline control injections were administered on intervening days. All injections of AMPH were given i.p. and the drug or saline condition was randomised across the rats within each group. Thus half the rats were given saline on days 1, 3 and 5 and the other half on days 2, 4 and 6. The dose of drug was individually randomised across the drug days. Following injection rats were placed into the locomotor

cages and the session was run under the same conditions as in the previous trials. Ratings of behaviour were made every 10 min using a modified version of the Creese-Iversen scale (Kelly *et al.*, 1975). The scores were as follows:

- 0 Still/Asleep.
- 1 Generally active.
- 2 Active with bursts of stereotyped sniffing and rearing.
- 3 Stereotyped sniffing and rearing over a wide area.
- 4 Stereotyped behaviour in one place.
- 5 Bursts of stereotyped licking or gnawing.
- 6 Continual licking or gnawing.

In addition to making these ratings, notes relating to the actual behaviours observed were taken in order to subsequently backup. In some cases rats were removed from the locomotor cages before the end of the trial as it was observed that severe self-licking or gnawing was occurring. In cases of self-mutilation rats were placed next to the observer in a plastic cage with a smooth floor covered with a layer of sawdust for closer observation and to prevent further injury.

Testing using APO began some 5 weeks after the AMPH trials had been completed, allowing any possible residual drug effects to be overcome. The rats were re-assigned into groups, taking care that they were not being tested in the same cages as under AMPH, thus controlling for drug/place conditioning. They were re-habituated to baseline in the locomotor cages for 4 days before testing with APO began. Testing again lasted for 6 days using 3 different doses of APO separated by 3 saline days: injections were all s.c. The doses of APO were 0.1, 1.0, and 3.0 mg·kg⁻¹ dissolved in 0.02% ascorbate-saline (to inhibit oxidation; ascorbate-saline was also used for the control injections). The method of

drug/saline allocation and the experimental procedure was identical to that used under AMPH.

Histological Procedures

Rats were sacrificed between 110 and 127 days after surgery. Care was taken to ensure that sacrifices from each group were spread evenly over this period. Every fifth section was processed for immunohistochemistry (TOH-positive and ChAT-positive neurones), enzyme histochemistry (NADPH-diaphorase) and Nissl substance (cresyl violet).

Statistical Analysis

All data were analysed parametrically using ANOVA or T-tests and *post hoc* analysis was carried out where necessary using Tukey's method for multiple comparisons. All the locomotor data were square-root transformed to reduce variance (Winer, 1971). Stereotypy ratings were analysed using the Fisher Exact Probability Test (Siegel, 1956).

Results

Histological Analysis

Ibotenate produced discriminably different lesions in the PPTg and DpMe (Figures 9:1, 9:2, 9:3 and 9:4). Table 9:1 shows the general damage summary and average lesion volume assessed in Nissl-stained sections. Following ibotenate infusions to the PPTg there was substantial loss of ChAT-positive and diaphorase-positive cells from this area. This was not observed following control injections or DpMe lesion (Fig. 9:3). Mean losses in the PPTg-lesioned group were 83.78% (SE=3.21) for ChAT-positive neurones and 78.91% (SE=3.26) for diaphorase-positive neurones. Only 3 DpMe-lesioned animals showed any cell loss in the PPTg. This was never bilateral and never estimated as more than 30% using either histochemical technique. A highly significant correlation coefficient between cell counts for

Figure 9:1

Representative sections re-drawn from the atlas of Paxinos and Watson (1986) illustrating the smallest and largest lesion sizes for (A) PPTg and (B) DpMe groups.

Figure 9:2

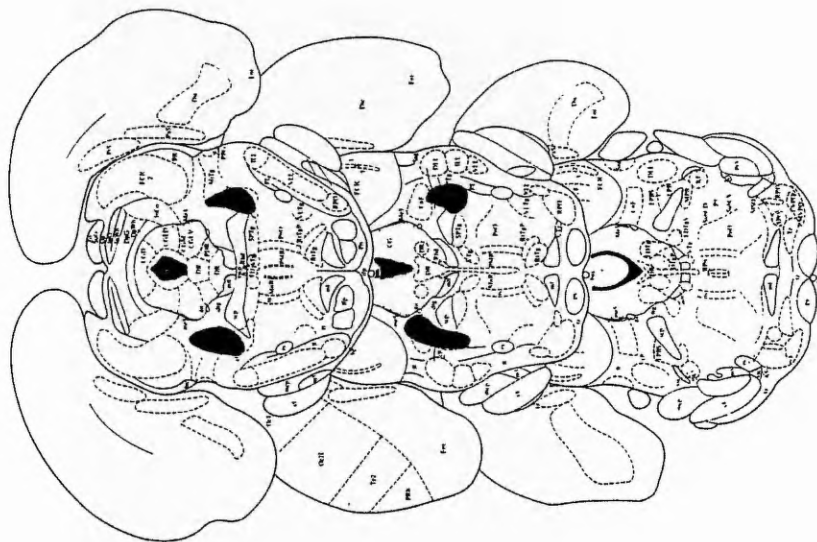
The figure shows a bilateral injection site for the PPTg lesion (arrows indicate the injection site on each side). The section is stained for Nissl substance. Aq: aqueduct anterior to 4th ventricle; CG: central gray; DR: dorsal raphe; scp: superior cerebellar peduncle; SPTg: subpeduncular tegmental nucleus. Scale bar: 0.25 mm.

Figure 9:3

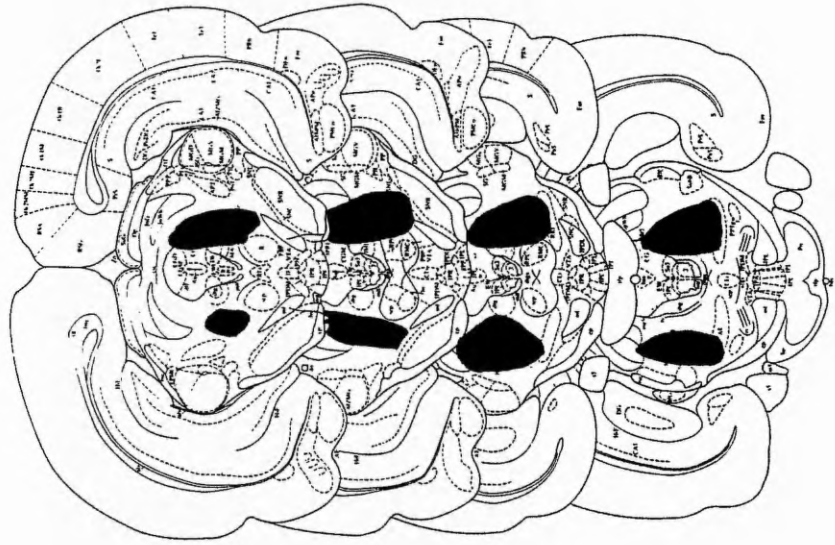
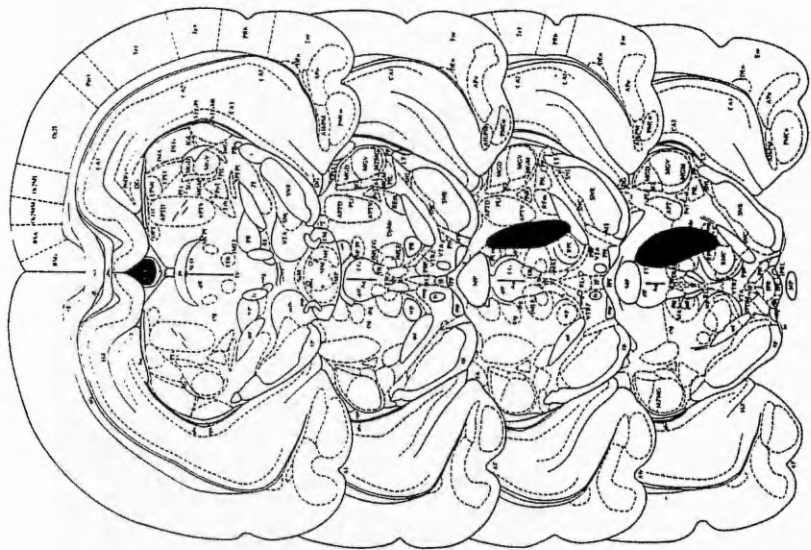
(A) Example of a section stained for NADPH-diaphorase showing intact PPTg in a DpMe-lesioned rat. Large intensely stained diaphorase-positive neurones are clearly visible. Scale bar: 0.1 mm; (B) Example of a section stained for NADPH-diaphorase at the site of the PPTg lesion. Only 2-3 cells remain in the region of the PPTg, while intensely stained cells of the SPTg are clearly undamaged. Scale bar: 0.1 mm; (C) and (D) Parallel sections to A and B stained for ChAT immunohistochemistry. Neurones in this region show excellent ChAT-positive staining. scp marks the superior cerebellar peduncle. Scale bars: 0.1 mm.

Figure 9:4

(A) Site of DpMe immediately dorsal to substantia nigra (SN) in a section stained for Nissl substance in a DpMe-lesioned rat. There is clear infiltration of microglia within the DpMe and calcification bordering the edge of the lesion site (arrows). R marks the magnocellular neurones of the red nucleus. Scale bar: 0.2 mm. (B) Identical site to A in a PPTg-lesioned rat. In this case the DpMe is clearly intact, and there are small calcium-rich crystals visible in the region of SN (marked by arrows) delineating the most anterior portion of the PPTg lesion. Scale bar: 0.2 mm.

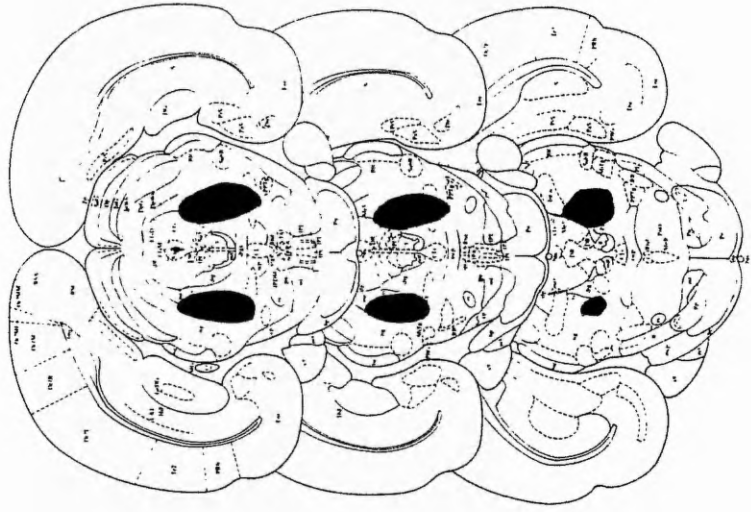


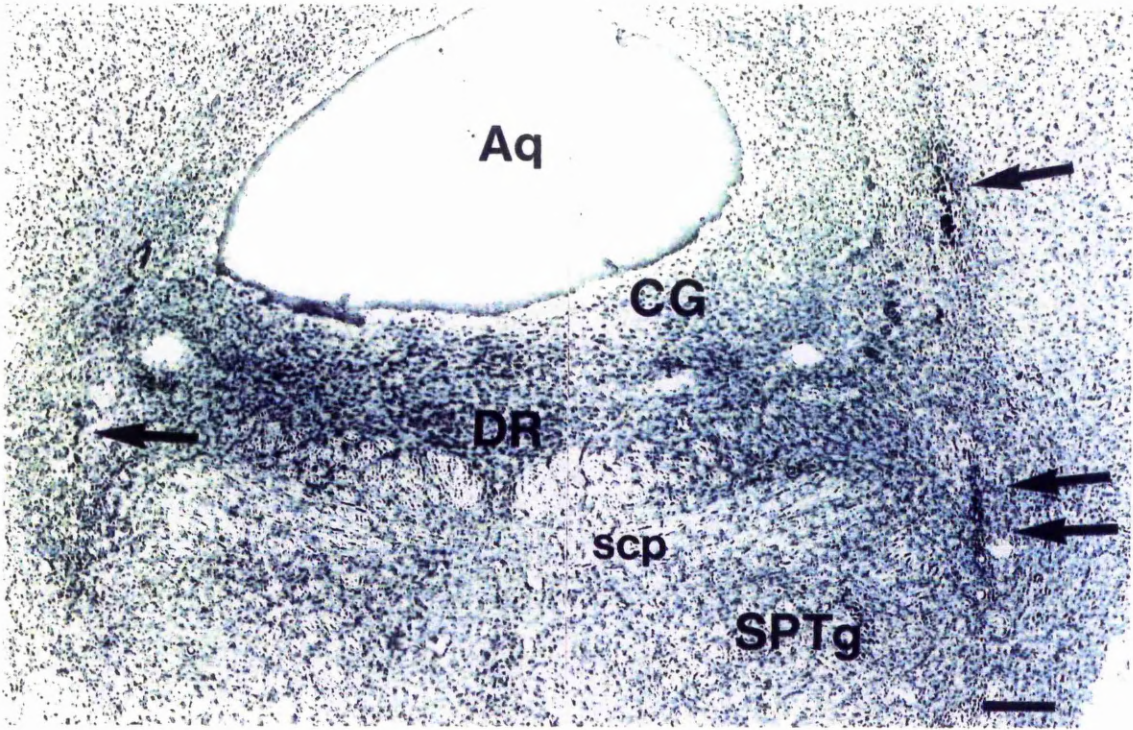
B

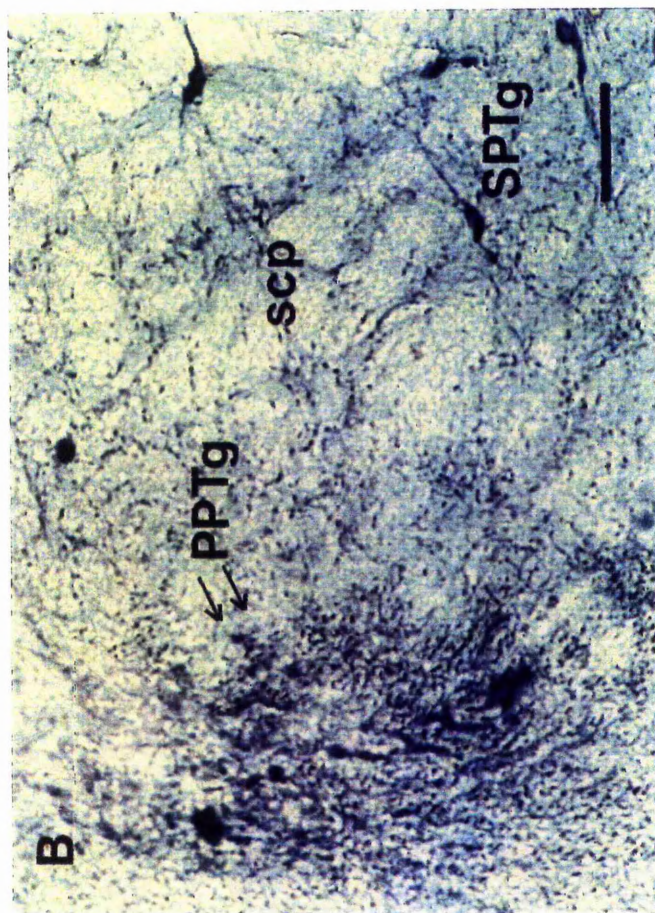
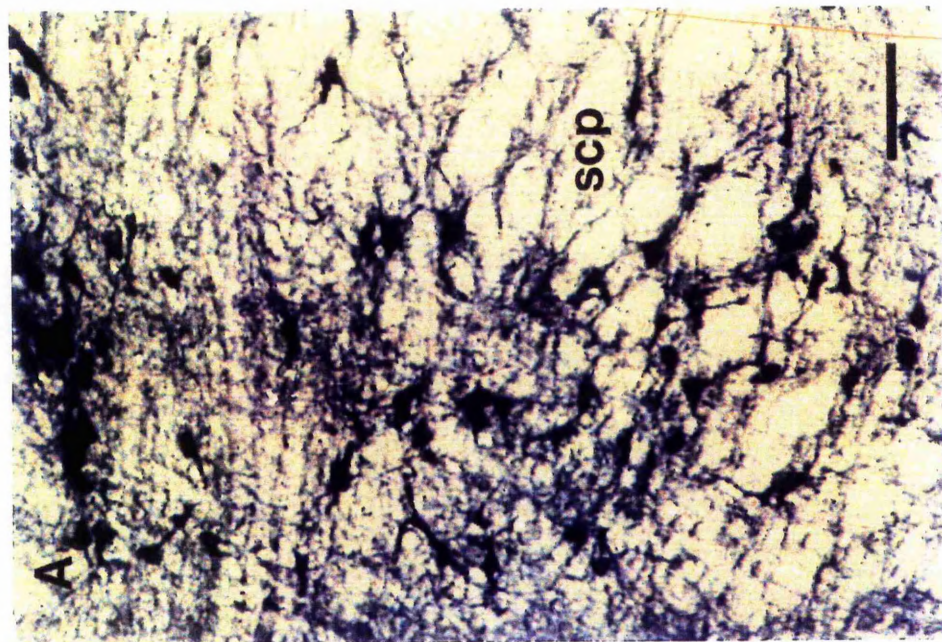


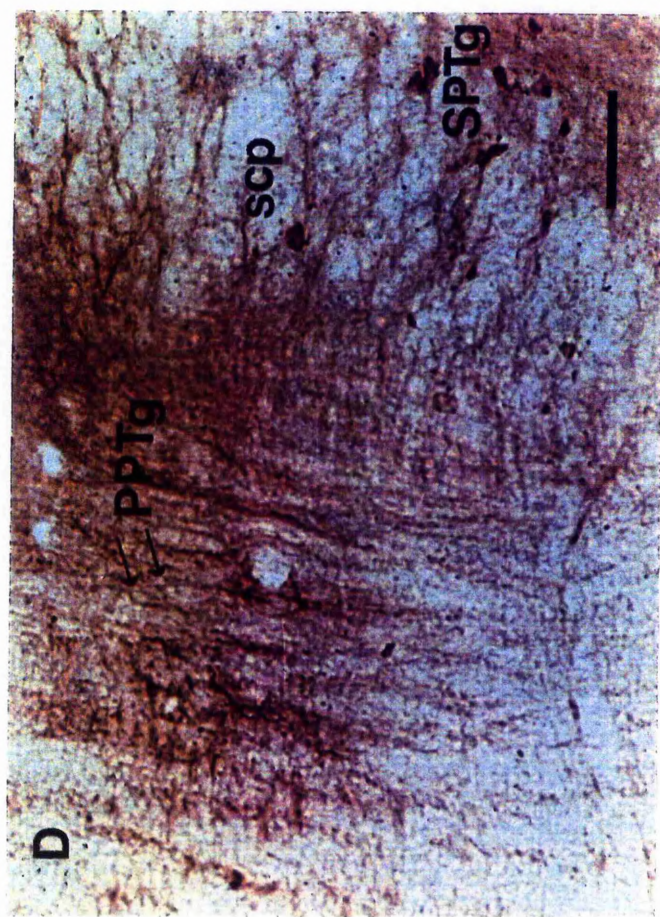
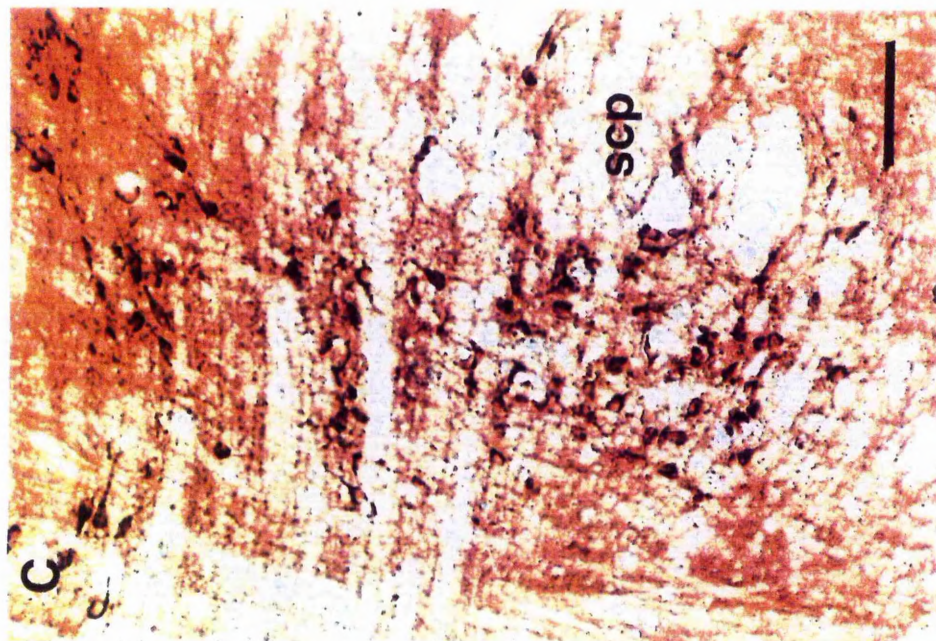
Smallest lesion

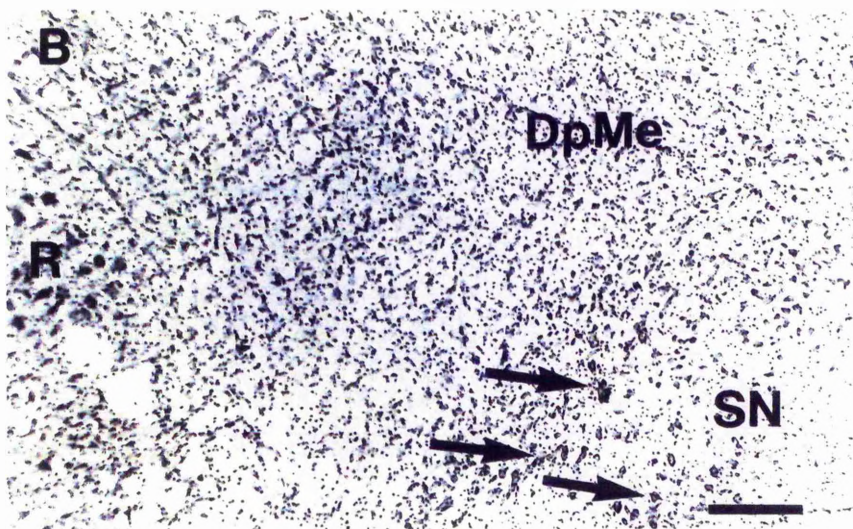
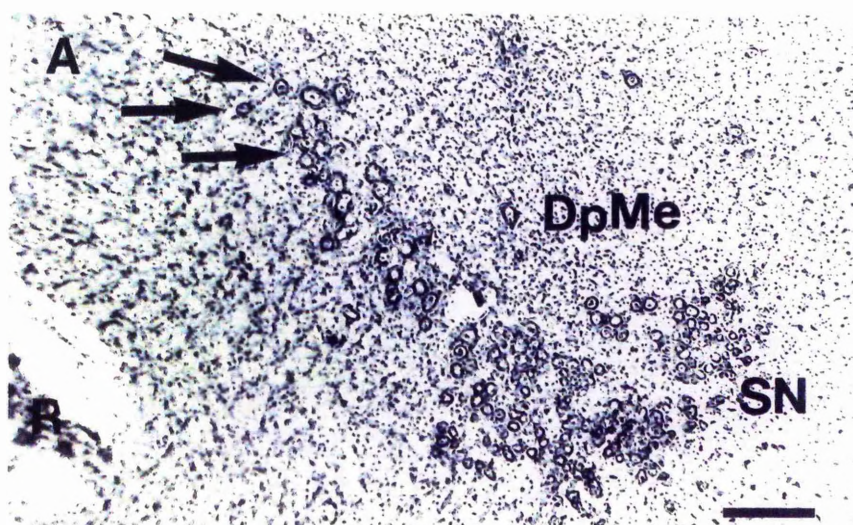
Largest lesion











ChAT and diaphorase was found (Pearson's $r=0.89$, $p<0.001$), suggesting that neurones stained for NADPH-diaphorase were predominantly cholinergic.

Differences between the lesion sites in respect of general neuronal damage were assessed by Nissl staining and TOH-positive neuronal loss (Table 9:1). As well as damage in the PPTg, ibotenate in this structure affected a large portion of the cuneiform nucleus and retrorubral field in all rats. It also caused minor damage to the superior cerebellar peduncle in 4 rats (although severe in 1 rat); 1 rat showed very minor damage to the subpeduncular tegmental nucleus (Ch5 diffuse interstitial component [Mesulam *et al.*, 1989]); and 2 rats showed slight loss in the rubrospinal tract. There was only marginal damage throughout the group to microcellular tegmental nucleus and the dorsal and central tegmental tracts which run through the region of the DpMe. Damage in the region of the SC was only ever caused by insertion of the injection needle, not by ibotenate. Ibotenate infusions to the DpMe produced a very different profile of damage. While in this group PPTg neuronal loss was never more than 30% (in 3/8 rats, and only unilaterally) damage to the DpMe and to the dorsal and central tegmental tracts was always severe, with minor damage to the lateral tegmental tracts. Neuronal loss in the retrorubral field, lying directly ventral to the DpMe, was also extensive although it was never completely destroyed. Four rats had very minor damage in the paratrochlear nucleus, while in 1 rat this was almost entirely removed. Loss in the central gray and SC was consistently small. Occasional unilateral damage (maximum score of 30%) occurred in some structures: the medial longitudinal fasciculus in 3 rats, the posterior commissure in 1 rat, and the ZI in 1 rat; the modal scores for these structures was no damage at all. In both lesion groups damage to the substantia nigra was principally unilateral and always less than 30%. The size of the damaged area in the DpMe-lesioned group was approximately twice that of the PPTg-lesioned group ($t=5.92$ $df=22$ $p<0.001$; see Table 9:1).

Table 9:1

A summary of damage and average lesion volumes (* $p < 0.001$ compared to PPTg) computed from the cresyl violet sections for each group. Damage was identified by glial infiltration and degeneration of neuronal somata, and a summary of affected structures for every animal estimated. The modal damage score in each structure is tabulated. The key is as follows:

ND	No damage
x	< 30% cell loss
xx	30 - 60% cell loss
xxx	60 - 90% cell loss
xxxx	> 90% cell loss

Lesion placement:	PPTg	DpMe
Damage assessment: (modal scores)		
Cuneiform nucleus	xx	ND
Superior cerebellar peduncle	xx	ND
Pedunculopontine tegmental nucleus	xxxx	x
Microcellular tegmental nucleus	xx	ND
Subpeduncular tegmental nucleus	ND	ND
Paratrochlear nucleus	ND	xx
Dorsal tegmental tract	xx	xxx
Central tegmental tract	xxx	xxxx
Lateral tegmental tract	ND	xx
Deep mesencephalic nucleus	xx	xxxx
Central gray	ND	x
Superior colliculus	track	xx
Rubrospinal tract	ND	ND
Retrobulbar nucleus	ND	ND
Retrobulbar field	xxx	xxx
Substantia nigra pars compacta	ND	ND
Lesion volume (mm³): (mean \pm SE values)	2.062 ± 0.18	4.065* ± 0.26

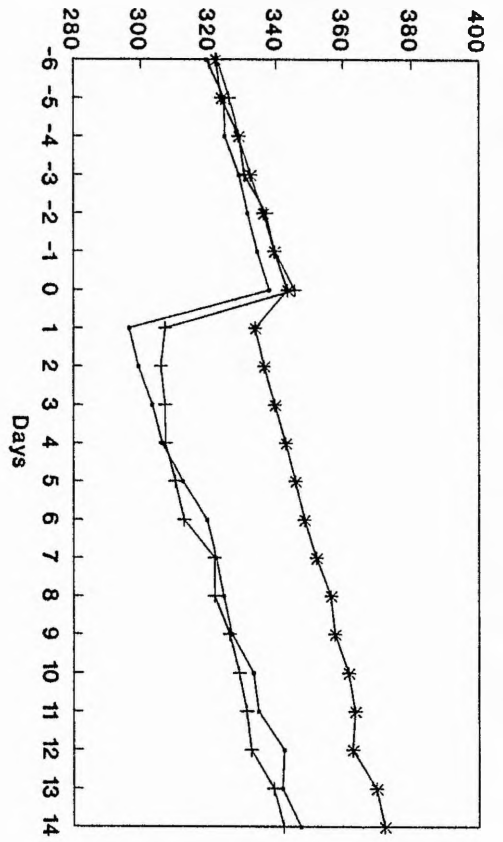
Body Weight, Spillage, Food and Water Intake

Figure 9:5 shows the average body weight, water intake, food intake and food spillage of each group for 7 days before and 14 days after surgery (taking, for the PPTg-operated rats, the second day of surgery as "surgery" and the 7 days before the first day of surgery for the pre-operative data). Analysis of the pre-operative data revealed no significant effects of group for body weight ($F=0.09$ $df=2,20$), spillage ($F=0.26$ $df=2,20$), food intake ($F=0.7$ $df=2,20$) or water intake ($F=3.22$ $df=2,20$). Analysis of post-operative data for body weight revealed significant main effects of lesion ($F=8.17$ $df=2,20$ $p<0.005$), days ($F=101.7$ $df=13,260$ $p<0.001$) and a significant group by days interaction ($F=1.9$ $df=26,260$ $p<0.01$). *Post hoc* analysis showed that this was attributable to both the PPTg- and DpMe-lesioned groups losing significantly more weight than controls following surgery. Recovery in the lesioned rats paralleled that of controls: both PPTg- and DpMe-lesioned rats were different to control every day ($p<0.001$) but never to each other ($p>0.42$ Day 1; $p>0.97$ thereafter). Post-operative analysis of food intake revealed significant main effects of lesion ($F=9.13$ $df=2,20$ $p<0.005$), days ($F=47.93$ $df=13,260$ $p<0.001$) and a lesion by days interaction ($F=4.73$ $df=26,260$ $p<0.001$). *Post hoc* analysis showed that the differences lay in the reduced food intake of both lesion groups compared to controls over the first 3 days after surgery and PPTg-lesioned group compared with control on post-operative day 4 (all $p<0.005$). From the fifth day after surgery on there were no significant differences in food intake between the groups. For food spillage, ANOVA revealed a significant effects of lesion ($F=6.45$ $df=2,20$ $p<0.01$) and days ($F=3.76$ $df=13,260$ $p<0.001$) but no significant interaction ($F=1.44$ $df=26,260$). *Post hoc* analysis revealed that only the DpMe-lesioned rats spilled more than controls ($p<0.05$). Analysis of water intake indicated a significant main effect of lesion ($F=7.99$ $df=2,20$ $p<0.005$) and days ($F=7.39$ $df=13,260$ $p<0.001$) but no interaction ($F=0.8$ $df=26,260$). *Post hoc* testing showed that the DpMe-lesioned rats drank more than those with PPTg

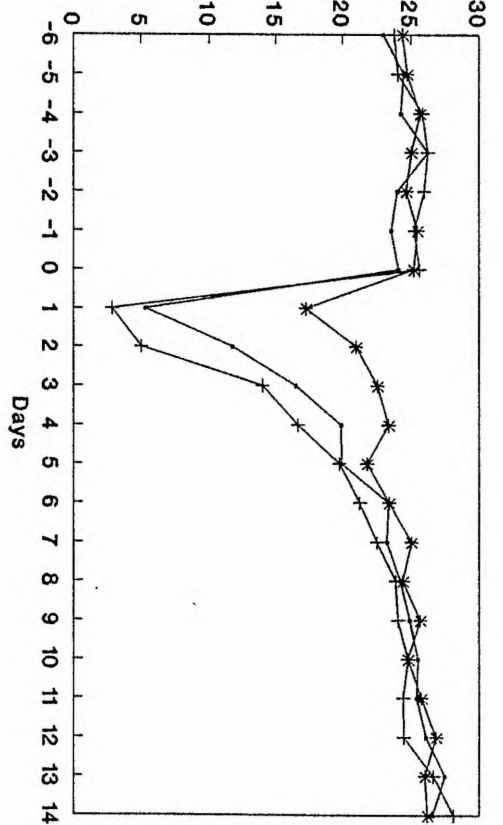
Figure 9:5

The average daily body weight, food intake, food spillage, and intake of water per group for seven days pre-operatively (negative numbers) and fourteen days post-operatively (positive numbers). Zero marks the day of surgery.

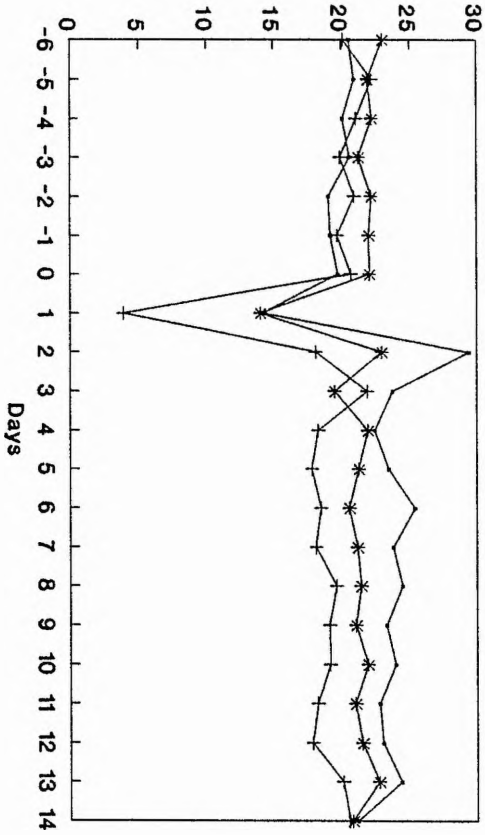
Body weight/g



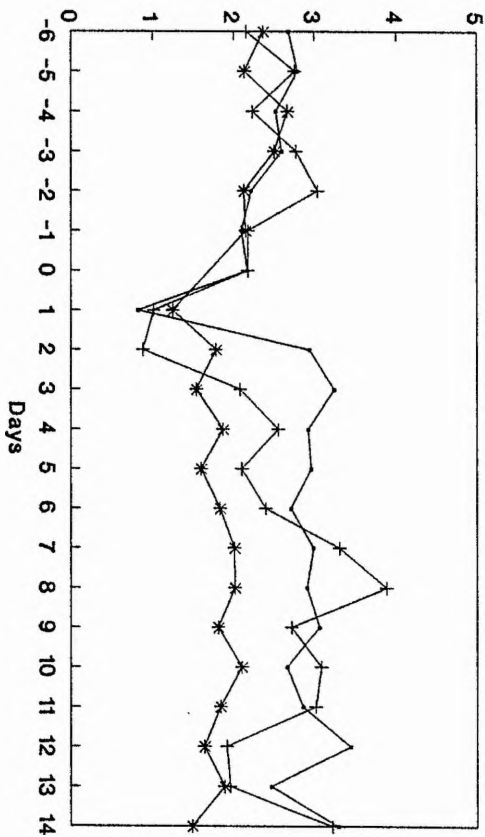
Food intake/g



Water intake/g



Food spillage/g



— DpMe-lesioned

+ PPtG-lesioned

* Control

lesions ($p < 0.01$) although neither were different to control. The differences in water intake between the groups never exceeded 5 ml.

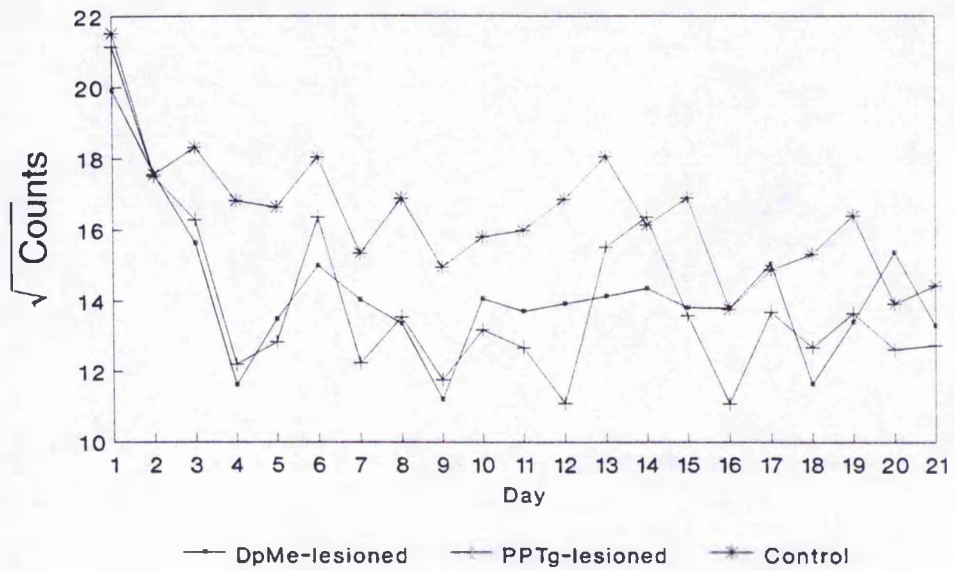
Spontaneous and Drug-induced Locomotion

Figure 9:6A shows the average spontaneous locomotor activity for each group over the 21 day test period. A significant effect of days was found ($F = 10.72$ $df = 20, 400$ $p < 0.001$), but there were no significant main effects of lesion ($F = 2.98$ $df = 2, 20$) or a lesion by days interaction ($F = 1.26$ $df = 40, 400$) indicating that baseline locomotor activity was unaffected by the loss of either the PPTg or DpMe. Figures 9:6B and 9:6C show the average locomotor activity in the groups following different doses of AMPH and APO. The 3 saline values in each group were collapsed as there were no significant differences between them in either drug analysis (p not less than 0.943). For AMPH, two separate analyses of lesion by dose were carried out: (i) an ANOVA excluding the PPTg group completely and (ii) an ANOVA including all groups (PPTg-, DpMe-lesioned and controls) but excluding the 3.0 and 5.0 $\text{mg} \cdot \text{kg}^{-1}$ doses. The PPTg-lesioned group were excluded in the first case because many of these rats had to be removed from the cages after treatment with both 3.0 $\text{mg} \cdot \text{kg}^{-1}$ and 5.0 $\text{mg} \cdot \text{kg}^{-1}$ AMPH to prevent self-mutilation (see below). In analysis (i) ANOVA revealed a significant main effect of dose ($F = 18.64$ $df = 3, 48$ $p < 0.001$) but not lesion ($F = 0.39$ $df = 1, 16$) or a lesion by dose interaction ($F = 1.39$ $df = 3, 48$). *Post hoc* analysis revealed that after 1.5 $\text{mg} \cdot \text{kg}^{-1}$ AMPH locomotor activity was increased more than at all other doses ($p < 0.001$ vs. saline and 5.0 $\text{mg} \cdot \text{kg}^{-1}$; $p < 0.05$ vs. 3.0 $\text{mg} \cdot \text{kg}^{-1}$). The 3.0 $\text{mg} \cdot \text{kg}^{-1}$ dose was also significantly different to saline ($p < 0.001$). In analysis (ii) there was again no main effect of lesion ($F = 0.67$ $df = 2, 20$) and no lesion by dose interaction ($F = 1.58$ $df = 2, 20$). All the groups were significantly more active after injection of 1.5 $\text{mg} \cdot \text{kg}^{-1}$ AMPH than saline ($p < 0.001$). For APO, analysis revealed no significant effect of dose ($F = 2.47$ $df = 3, 60$) or lesion ($F = 3.13$ $df = 2, 20$) although there was a significant lesion by dose interaction ($F = 4.01$ $df = 6, 60$ $p < 0.005$). *Post hoc* analysis

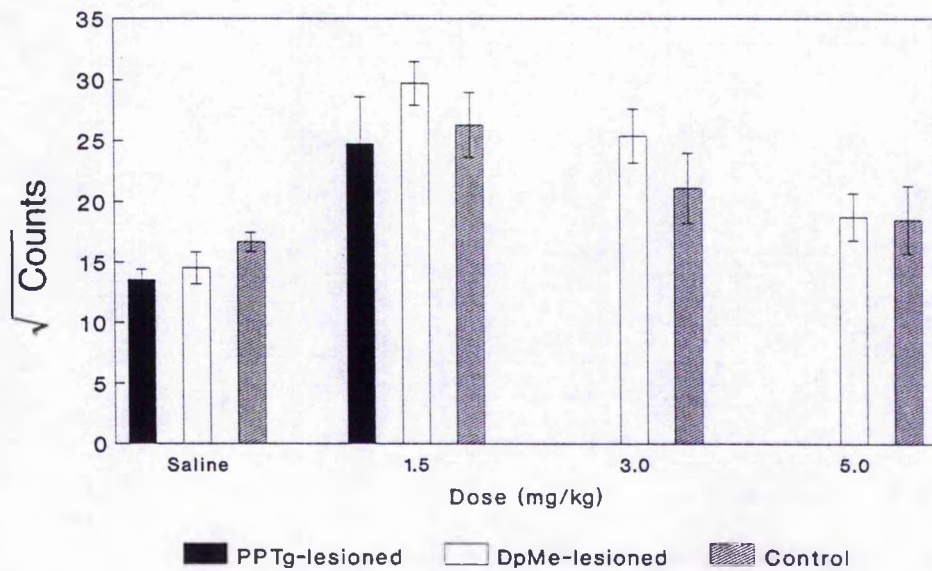
Figure 9:6

(A) The average daily locomotor activity for each group over a 21 day test period. Data were square-root transformed as recommended by Winer (1971). It should be noted that the scale of the y-axis is different to that in B and C. (B) The average locomotor activity for each group following different doses of amphetamine. Note that the PPTg ibotenate group are only included at the saline and $1.5 \text{ mg}\cdot\text{kg}^{-1}$ points in the amphetamine testing. At the two higher doses the rats were removed from their cages to prevent self-mutilation and locomotor counts were not obtained. Data were square-root transformed. (C) The average locomotor activity for each group following different doses of apomorphine. Data were square-root transformed.

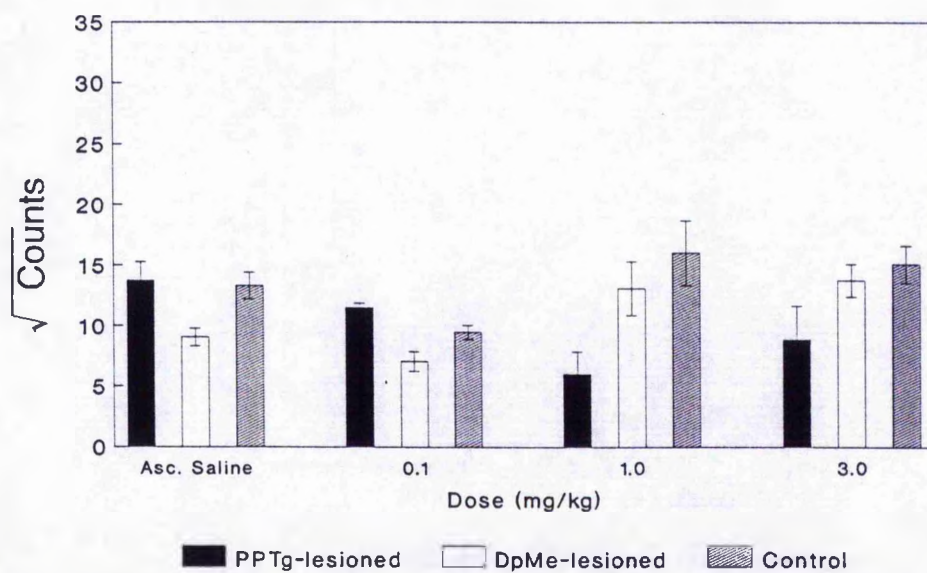
Baseline Locomotion



Amphetamine



Apomorphine



revealed that at $1.0 \text{ mg}\cdot\text{kg}^{-1}$ the PPTg-lesioned group were hypoactive compared with saline and with control rats at $1.0 \text{ mg}\cdot\text{kg}^{-1}$ (both $p < 0.05$).

Drug-induced Stereotypy

Table 9:2 shows the frequency with which particular scores on the Creese-Iversen scale were observed in each group after different doses of AMPH or APO. The scores are presented as percentages in the table for clarity while the analysis by Fisher's exact probability test compares modal scores calculated for each rat. At $1.5 \text{ mg}\cdot\text{kg}^{-1}$ AMPH all rats, regardless of lesion, displayed a general increase in activity with bursts of stereotyped sniffing and rearing, sometimes over a wide area of the cage. The scores for each group were spread evenly across the lower end of the Creese-Iversen scale and were not significantly different (Fisher exact comparing modal Creese-Iversen scores of 1-2 vs. 3-4: DpMe vs. PPTg; DpMe vs. control; PPTg vs. control: all $p > 0.05$). At both the higher doses of AMPH however, differences between the groups appeared, as Table 9:2 makes clear. The PPTg-lesioned rats showed a marked shift in their response at $3.0 \text{ mg}\cdot\text{kg}^{-1}$: significantly more rats in this group showed stereotypies which included biting than in either the DpMe or control groups (modal scores of 2-4 vs. 5-6: $p = 0.03$ and $p = 0.02$ respectively) while DpMe and control groups were not different to each other ($p = 0.23$). In fact only PPTg lesioned rats scored 5 or 6 at this dose; none of the DpMe lesioned or control rats scored more than 4. At $5.0 \text{ mg}\cdot\text{kg}^{-1}$ the PPTg-lesioned group was different to control (modal scores of 2-4 vs. 5-6: $p = 0.02$) but not to the DpMe-lesioned group ($p = 0.22$). Some biting was observed at this dose from all of the PPTg-lesioned rats but only some DpMe-lesioned rats, although the latter were not statistically different to the control group ($p = 0.16$) where no biting was observed. The stereotyped biting, gnawing and licking shown by the PPTg-lesioned rats was predominantly directed at their forepaws. Vacuous gnawing and licking of the cage floor was also occasionally noted. In severe cases moving the rat from the test cage to a plastic cage with a smooth floor was necessary: this was

sometimes enough to prevent the recurrence of self-gnawing which was replaced by vacuous gnawing/licking. If the self-gnawing was not reduced by removal from the test cage a pen barrel was sufficient to intercept the behaviour: the rats would continuously lick rather than gnaw this.

Under APO the PPTg-lesioned rats again showed a clear shift in the dose response curve compared with the other groups. Following $0.1 \text{ mg}\cdot\text{kg}^{-1}$ they were more active than the other groups (comparing modal scores of 0 vs. 1-2: PPTg vs. DpMe, $p=0.001$; PPTg vs. control, $p=0.005$). At $1.0 \text{ mg}\cdot\text{kg}^{-1}$ both PPTg and DpMe groups showed higher stereotypy scores than controls (comparing modal scores of 2-4 vs. 5-6: PPTg vs. control, $p=0.003$; DpMe vs. control, $p=0.007$) and were not statistically different to each other (PPTg vs. DpMe, $p=0.08$). Similarly at $3.0 \text{ mg}\cdot\text{kg}^{-1}$ the PPTg rats gnawed and licked more than the controls (comparing modal scores of 2-4 vs. 5-6: $p=0.02$) but were not statistically different to the DpMe group. However, neither the control nor the DpMe rats ever scored 6 following 1.0 and $3.0 \text{ mg}\cdot\text{kg}^{-1}$ while more than 20% of the scores collected for the PPTg rats at each of these doses was 6. APO, unlike AMPH, produced gnawing and licking that was predominantly directed at the cage bars. However there was more behavioural variation in the PPTg group following APO than AMPH: there was more licking rather than gnawing and the target for the behaviour was more arbitrary. While the cage-bars were a common target of gnawing for all rats in this group, rats would also lick forepaws and hindpaws and penile grooming was also observed. One PPTg-lesioned rat began to gnaw fiercely at its tail following both $1.0 \text{ mg}\cdot\text{kg}^{-1}$ and $3.0 \text{ mg}\cdot\text{kg}^{-1}$ APO and had to be prevented from doing so by offering it polystyrene chips to gnaw. The focus of gnawing and licking for this rat then changed from tail/paws to polystyrene chips/cage and back again, though the actual activity was continuous.

Discussion

These data show that lesions in the PPTg and DpMe have discriminably different effects, measured histologically and behaviourally. There were no gross regulatory deficits following either lesion although DpMe-lesioned rats showed a small but significant increase in drinking and spilled more food than controls. There was no locomotor impairment in either lesion group: spontaneous locomotion and that stimulated by low doses of AMPH and APO was unaffected by either PPTg or DpMe lesions. Stereotyped behaviour was however affected: in PPTg- but not DpMe-lesioned rats systemic AMPH elicited biting and the dose-response to APO was shifted to the left. The biting exhibited by PPTg-lesioned rats following AMPH was predominantly directed at their own forepaws, while oral behaviour stimulated in this group following APO was directed variously at the cage bars, paws, tail or penis. These data suggest that the PPTg may play an important role in mediating outflow from the CPu but not the NAcc.

The present data demonstrate that both DpMe and PPTg have a role in motor control but that it is possible to discriminate between them. The increased water intake in the DpMe-lesioned group might have been predicted as the DpMe receives a monosynaptic input from the ZI (Shammah-Lagnado *et al.*, 1985), a structure thought to be involved in regulatory drinking (Rowland *et al.*, 1979). Alternatively the fact that this group of rats also increased food spillage suggests that they had a more general oral motor deficit: both food spillage and the apparent increase in drinking may reflect increased spillage rather than altered consumption. This is a more likely explanation than a regulatory modification of water intake given that the increase in drinking was very small. In the cat, increased numbers of tongue protusions have been observed following administration of the GABA_A antagonist picrotoxin to an area similar in location to the ventral portion of the lateral tegmental tract in the DpMe (Spooren *et al.*, 1993). This region is thought to be an output station for the subcommisural part of the GP, an area from which

tongue protrusions and tic-like contractions of the facial, eye and ear muscles can be stimulated (Cools *et al.*, 1989; Spooren *et al.*, 1993). The present data certainly suggest that at least some of the output channels which run through the DpMe contain information related to tongue use and it appears that the neurones in question may be in the ventrolateral portion of the nucleus. Regardless of the nature of the DpMe deficit however, it is important to recognize that lesions to the DpMe and PPTg did not have the same effects in this regard.

PPTg as a striatal output station

The presence of the PPTg is often now acknowledged in accounts of striatal outflow (for instance, Gerfen *et al.*, 1990; Mitchell *et al.*, 1989) but its precise role is rarely, if ever, commented upon. Two sound reasons for this are the lack of a clear definition concerning the constitution of the PPTg (see Chapter 1) and the relative lack of clear experimental evidence concerning its function (see Chapter 4). We have tried to address both of these issues using a well-worked out lesion technique with an appropriate anatomical control procedure.

Locomotion and the NAcc. The data presented here argue in favour of a role for the PPTg in mediating striatal outflow, but indicate that it may be more important in mediating CPU rather than NAcc output. Indeed, a surprising aspect of the present data is the absence of any effect of PPTg lesions on locomotion: neither spontaneous nor drug-induced locomotion (known to be dependent on DA systems in the NAcc) was affected. DpMe lesions were also without effect in this regard. Previous experiments concerning the role of the PPTg in mediating locomotor outflow from the NAcc have been contradictory: for instance some authors have reported a blockade following procaine infusion into this area (Brudzynski and Mogenson, 1985) while others that ibotenate lesions had no effect in reducing the "supersensitive" locomotor response to apomorphine in the 6-OHDA-lesioned NAcc (Swerdlow and Koob, 1987). Although the early association between the

PPTg and the mesencephalic locomotor region (Shik *et al.*, 1966) has recently been weakened considerably (Eidelberg *et al.*, 1981; Coles *et al.*, 1989; Shojania *et al.*, 1992), a role for the PPTg in some form of locomotor activity should not be completely discounted. It has been argued recently by Garcia-Rill (1991) however that electrical stimulation of this area could synchronize in an artificial manner the firing of rhythmogenic neurones in the PPTg. Behaviour produced under such circumstances would tend towards a lowest common denominator: probably locomotion. Indeed, probable functional links between the NAcc and PPTg have been demonstrated by the use of reward- and aversion-related tasks (Chapter 4). For instance, there are reports of attenuating effects of ibotenate PPTg lesions on morphine-induced conditioned place preferences in naive rats (Bechara and van der Kooy, 1989) and the ability of ibotenate or kainate PPTg-lesioned rats to use a warning buzzer/light stimulus to avoid footshock was deficient (Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992). As discussed in Chapter 4, such functional links between the ventral striatum and the PPTg may not actually mediate reward or locomotion *per se*, but instead may play a role in the integration of locomotor output signals or motivationally significant stimuli with other aspects of motor output.

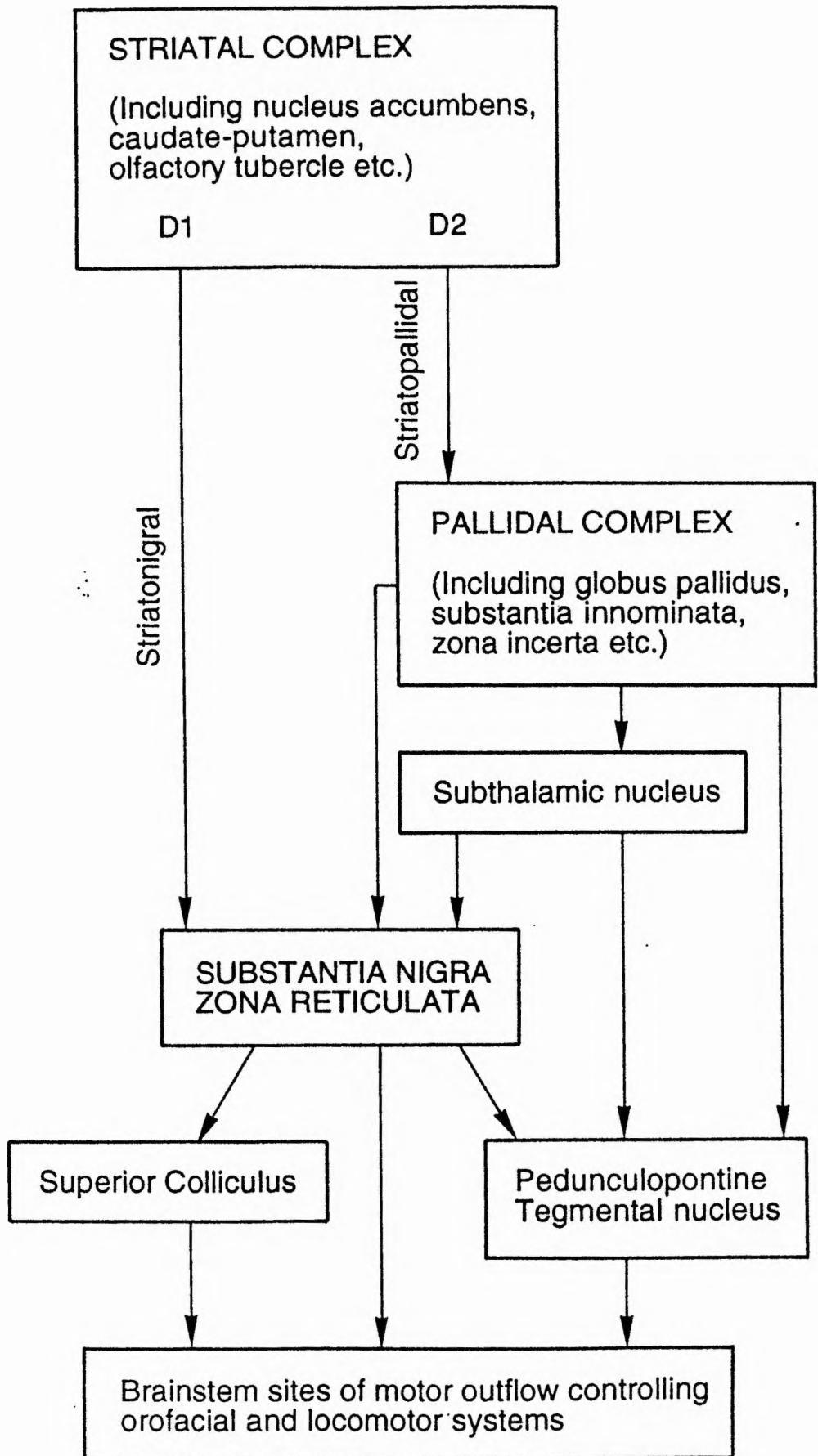
Biting and the CPu. Biting behaviour is almost never observed after systemic AMPH, but is after APO (Fray *et al.*, 1980). These drugs have different actions: AMPH blocks re-uptake and promotes release of catecholamines (Butcher *et al.*, 1988) and might be expected to affect indirectly both D₁ and D₂ DA receptors. APO by contrast is a relatively selective direct D₂ DA receptor agonist. The locomotor stimulant properties of both of these drugs depends on an action within the NAcc; stereotypies on the CPu (Kelly *et al.*, 1975). Pure biting and gnawing activity can appear after direct stimulation of a discrete portion of the ventrolateral CPu with AMPH: Kelley and her colleagues (Kelley *et al.*, 1988) have demonstrated that microinjection of AMPH (20 µg / 0.5 µl) into the ventrolateral

CPu produces vigorous biting of the forepaws. Concurrent stimulation of D_1 and D_2 DA receptors is necessary for the expression of intense oral stereotypies: D_1 stimulation alone produces no observable behavioural change, while D_2 stimulation increases licking, sniffing and general mouth movements in a dose-dependent manner, though not gnawing or biting (Delfs and Kelley, 1990). While it could be argued that in fact the effects of these microinjections are mediated by drug diffusing from the ventrolateral CPu into the NAcc there are reasons for believing that this is not so. First, the stereotypies induced by systemic APO that often include biting, licking and gnawing are blocked by 6-OHDA lesions of the CPu but not NAcc (Kelly *et al.*, 1975); second, discrete lesions of this area of the CPu impair tongue use (Pisa, 1988); and third, this area of the CPu in the rat may be homologous to the primate ventral putamen, selective stimulation of which elicits orofacial movements (Alexander and De Long, 1985). Input to this region is from structures known to be important in taste perception (amygdala, inferior temporal cortex, perirhinal cortex: Van Hoesen *et al.*, 1981; Russchen *et al.*, 1985). The primary output is to the dorsolateral part of the SNr (Tulloch *et al.*, 1978; Gerfen, 1985; Von Krosigk *et al.*, 1992) which connects with descending GABAergic neurones projecting to the parvicellular reticular formation, a region which has direct links with orofacial motor nuclei (Travers and Norgren, 1983).

Figure 9:7 presents a simplified account of dorsal striatal output. Simplification should not however obscure the fact that the internal organization of the striatum is fiendishly complicated (Gerfen, 1989; Gerfen, 1992; see also Chapter 3) and the two major outputs, striatopallidal and striatonigral, are not homogeneous. In Figure 9:7 the pallidal complex includes not only the GP but also the substantia innominata and ZI. Pallidal outflow is directed at the thalamus (left out of the Figure for the sake of clarity), SNr, STn and PPTg. The output of the SNr is directed at the PPTg, SC and brainstem sites of motor outflow and again, the thalamus. The relative degree to which the pallidum, SNr, PPTg and SC become

Figure 9:7

Diagram of the connections of striatal output neurones with SNr, PPTg, SC and brainstem sites of motor outflow. (See text for discussion.)



involved in mediating striatal output depends upon a number of factors. As specific portions of the striatum receive input from different portions of the cortex and limbic system, and as D_1 and D_2 DA receptors are generally located on striatonigral and striatopallidal neurones respectively (Gerfen *et al.*, 1990), so distinct pharmacological treatments will create disparate patterns of striatal outflow.

Orofacial behaviour such as biting is mediated by striatonigral output from the ventrolateral CPU to the dorsolateral SNr (Tulloch *et al.*, 1978; Gerfen, 1985), from where efferent neurones contact the lateral reticular formation, parvocellular reticular formation and related portions of the dorsal and ventral medullary reticular formations (Von Krosigk and Smith, 1991). These are the brainstem sites that project to orofacial motoneurones (Travers and Norgren, 1983). The dorsolateral SNr also projects to the rostral intermediate layers of the SC (Redgrave *et al.*, 1992) and it is clear that the nigrotectal pathway can regulate biting. Intranigral muscimol induces biting dependent on these neurones (Childs and Gale, 1983) while electrolytic lesions of the SC reduce AMPH stereotypy and APO-induced gnawing, but not stimulus-bound feeding (Pope *et al.*, 1980; Redgrave *et al.*, 1980; Dean *et al.*, 1984). It is also of interest that ibotenic acid lesions of the ZI - which has reciprocal connections with the SC (Shammah-Lagnado *et al.*, 1985) - or selective antagonism of ZI AMPA/kainate glutamate receptors inhibit AMPH- and APO-induced stereotypy but do not affect AMPH-induced locomotion (Supko *et al.*, 1992). Collicular involvement in orofacial activity presumably relates to visual guidance of behaviour.

The present data show however that orofacial activity is not just dependent on an axis running from the ventrolateral CPU, through the dorsolateral SNr to the SC and medullary motor outflow sites, but that the PPTg also has a role to play. The PPTg receives information from the SNr (Von Krosigk and Smith, 1990; Spann

and Grofova, 1991) as well as the pallidal complex (Moriizumi *et al.*, 1988; Takada *et al.*, 1988) and the descending output from the PPTg parallels that of the SC and SNr (Rye *et al.*, 1988; Garcia-Rill, 1991). But whether the PPTg receives information directly concerning orofacial activity or not is unclear. The demonstration by Delfs and Kelley (1990) that concurrent D₁ and D₂ stimulation is required to induce biting suggests that orofacial activities are not dependent exclusively on either striatonigral or striatopallidal output. It is therefore conceivable that some output concerning orofacial activity could reach the PPTg via striatopallidal neurones, via the SNr or by both routes. The excess licking and biting observed in this study suggests that such information would normally have inhibitory effects on orofacial behaviours.

However another alternative is that the PPTg may not receive information concerning orofacial output at all, but instead be in receipt of information about behaviours incompatible with orofacial movements. The data presented in Chapter 4 which relate to a role for the PPTg in the use of motivationally significant stimuli to direct behaviour suggest that the PPTg is in receipt of information relating to "appropriateness" of certain responses. Therefore it is possible that the PPTg normally gates firing in the pontine oral nuclei so that orofacial activity only occurs in pertinent situations. The absence of a locomotor deficit in PPTg-lesioned rats indicates that locomotion is also unlikely to be mediated there in a straightforward fashion although the PPTg may receive information relating to muscle tone (Kelland and Asdourian, 1989). Other behaviours stimulated by AMPH, such as sniffing and rearing (Lyon and Robbins, 1975) might also have some representation in the PPTg: hypotheses concerning competition between striatal outflow systems have been presented previously (Joyce and Iversen, 1984) but fundamental questions about such competition remain unanswered. Are there really identifiable streams of striatal output, each tagged with particular behavioural commands, or are different behavioural outputs represented by patterns of activity in striatal

outflow rather than being rigidly dependent on individual systems; and most important, where and how does selection concerning behavioural output finally occur?

Answers to these questions are not only of academic interest. The PPTg is affected in many clinical conditions in a variety of ways: in Parkinson's disease there is thought to be chronic inhibition of the PPTg by overactive pallidal outflow (Mitchell *et al.*, 1989), as well as loss of PPTg cholinergic neurones (Hirsch *et al.*, 1987; Zweig *et al.*, 1987; Jellinger, 1988; Zweig *et al.*, 1989; Halliday *et al.*, 1990a, 1990b). In schizophrenia striatal outflow can be altered by disturbances in the level of DA occurring both naturally and in response to medication. It is therefore of interest to note the frequency with which orofacial dyskinesias appear in these conditions, in experimental models as well as clinically. Data from early experimental work on Parkinson's disease describes the production of orofacial dyskinesias by L-Dopa in monkeys with tegmental lesions (Battista *et al.*, 1971). The intensity of observed oral movements depended on the dose of L-Dopa: at low doses (i.p. 30 mg·kg⁻¹) lip-smacking, tongue-rolling and chewing were observed, but as the dose was increased (i.p. 100-150 mg·kg⁻¹) grimacing was common and chorea-like movements of the head and limbs were also often present. More recently, the site of L-Dopa-induced dyskinesia has been investigated in the monkey (Crossman *et al.*, 1988). Injections of the GABA antagonist bicuculline (15 µg/µl) into the lateral segment of the GP and immediately adjacent regions induced choreic movements. In the mild/moderate form, these primarily involved the orofacial musculature, while at their most severe they took the form of limb movements which were practically indistinguishable from hemiballism.

Sacks (1991) also reported involuntary oral-motor dyskinesias which appeared as side-effects to L-DOPA therapy in several of his post-encephalitic patients. For instance, he wrote of Frances D: "her inclination to munch and gnaw grew greater

and greater, she would chew and over-chew her food, ... and in the absence of food would bite her lips or gnash her teeth" (pp. 50-51). Similarly Maria G. "showed violent out-thrustings of the tongue and a continual tonic protusion of the lips ... I gave her a pencil and paper, but she thrust them into her mouth and chewed them to pieces". After eating she "would have an irresistible urge to lick her plate, and stuff her fingers and utensils into her still chewing mouth". Such observations are not uncommon clinically and similar oral stereotypies have been observed in Tourette's syndrome and in schizophrenic patients. In Wilson's disease loss of control of the orofacial musculature and excessive drooling is observed (Walshe, 1986). The PPTg - and indeed the DpMe, loss of which appears to have produced an impairment in the acts of eating and drinking - may be implicated in these aspects of basal ganglia disorders.

Conclusions

The data presented in this Chapter demonstrate that:

1. The PPTg lesions developed in this laboratory (Rugg *et al.*, 1992) can be dissociated both histologically and behaviourally from lesions placed in the DpMe.
2. Lesions placed in the DpMe do not affect regulatory functions or the outflow of behaviour from the striatum. However, rats with DpMe lesions may have a small oral-motor deficit as demonstrated by the increased spillage of food, and perhaps water, from this group.
3. Lesions placed in the PPTg do not affect regulatory functions or the outflow of locomotor activity from the NAcc. However, PPTg-lesioned rats did display abnormal oral movements following systemic AMPH or APO, which implicates the PPTg in some form of modification of outputs from the CPu. This may be important in terms of the inappropriate behaviours observed from patients with diseases of the basal ganglia.

10. Outflow from the striatum: effects of PPTg lesions on responding for conditioned reinforcement elicited by stimulation of nucleus accumbens

Introduction

Release of DA in the NAcc by AMPH produces increases in locomotor activity and in the direction of motivationally significant behaviours. As a result of the clear anatomical links between the NAcc and PPTg-nCh, studies which have investigated the presumed links between these structures first implicated the PPTg as a locomotor centre (Brudzynski and Mogenson, 1985; Milner and Mogenson, 1988; Mogenson and Wu, 1988; Garcia-Rill *et al.*, 1990; Garcia-Rill, 1991; see Chapter 4) and second suggested that it might be an important site for the mediation of incentive-related behaviour (Bechara and Van der Kooy, 1989; Bechara and Van der Kooy, 1992a; Bechara and Van der Kooy, 1992b; Bechara and Van der Kooy, 1992c; Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992; see Chapter 4). Although there is growing evidence to demonstrate that the PPTg-nCh does not mediate outflow of either spontaneous locomotion (Dellu *et al.*, 1991; Chapter 9), that induced by systemic AMPH or APO (Swerdlow and Koob, 1987; Olmstead and Franklin, 1992; Chapter 9) or locomotor activity induced by AMPH injections directly into the NAcc (JS Dunbar and P Winn, unpublished observations), its role as an output station for the NAcc in the mediation of incentive-related behaviours is undisputed.

As discussed in Chapter 4, the PPTg-nCh appears to be essential for establishing incentive associations between novel stimuli so that they can be used to direct incentive-driven behaviours. However, the data which have been collected to investigate this phenomenon have not made it clear whether the PPTg-nCh is required specifically to form each individual stimulus-incentive association and use this to direct motor output towards the perceived reward, or whether its role is in forming simultaneous associations between several disparate stimuli and one type

of incentive. For example, in the formation of morphine place preferences (Bechara and Van der Kooy, 1989) did the PPTg-nCh enable naive rats to form a link between the morphine and reward so that they could use this association to approach the place where the reward occurred (and form a place preference), or did the PPTg-nCh enable these rats to link both the morphine and the compartment with the same motivational indicator? In the latter case, without a PPTg rats might still be able to link one component stimulus with one incentive (for example morphine with reward or place with reward) but not more than one component simultaneously with the same internal drive (morphine and compartment with reward). The place preference experiments which used morphine-dependent rats do not answer this query either: could these rats form place preferences because they have formed an incentive association (withdrawal alleviation = reward) previously to direct their motor output; or is it because they only have to link one stimulus component (the compartment) with a previously learned stimulus-reward connection?

A behavioural task which has not previously been used to investigate the role of PPTg-nCh neurones in the outflow of DA-mediated information from the NAcc is the facilitation of operant behaviour by AMPH in the conditioned reinforcement paradigm (Taylor and Robbins, 1984; Kelley and Delfs, 1991; Chapter 8). This procedure may be an ideal way to begin approaching the problem outlined above. In the training phase, an arbitrary stimulus (for instance, light/click of the food hopper) is paired in a Pavlovian manner with the presentation of a primary reinforcer (for instance, food). In the test phase, food is no longer presented but pressing one of two levers results in the presentation of the conditioned reinforcer (CR) (light/click), while pressing the other lever (NCR) has no programmed effect. Injections of AMPH into the NAcc dose-dependently increases responding for CR but not NCR (Taylor and Robbins, 1984; Kelley and Delfs, 1991; Chapter 8). Rats trained to associate the light/click stimulus with food presentation prior to making

lesions in the PPTg ought to retain this information after surgery (Bechara and Van der Kooy, 1989; Fujimoto *et al.*, 1992). Then in the test phase, if the PPTg-nCh is required to form each individual stimulus-incentive association, then lesioned rats should not be able to associate the lever-press with reward and therefore their acquisition of responding for CR should be blocked. If on the other hand the PPTg-nCh is required only where more than one novel stimulus must be simultaneously tagged to one incentive, then the acquisition of lever-pressing for CR should not be affected by the lesion.

While neurones in the PPTg-nCh may be important for the formation of associations between motivationally significant stimuli, interactions between the PPTg-nCh and the PPTg-Ch are also likely to be essential for integration of such associations back into basal ganglia and cortical circuitry (Chapter 4). The implication of this, that damage to PPTg neurones in either category would be sufficient to impair the use of motivationally significant stimuli, can be tested directly. In this laboratory, we have demonstrated that different types of excitotoxins infused into the PPTg result in distinct profiles of neuronal damage (Rugg *et al.*, 1992). Although there was little difference in the extent to which different excitotoxins such as ibotenate, quisqualate, NMDA, quinolinate and kainate destroyed PPTg-Ch neurones, they were distinguishable from each other in their relative destruction of PPTg-nCh neurones. In fact, quinolinate (QUIN) was most selective in its destruction primarily of PPTg-Ch neurones, while ibotenate (IBO) was the excitotoxin of choice for equal destruction of both neuronal populations. The observed histological differences between lesions made with QUIN and IBO were subsequently validated here in a reaching/grasping task (Dunbar *et al.*, 1992). Therefore in the CR test phase, if the PPTg-nCh is required to form the lever-press/reward association, then IBO lesions of the PPTg will impair responding; QUIN lesions may also impair responding to some degree as it does partially damage PPTg-nCh neurones, but a severe deficit in responding on

the lever would only be expected following QUIN if the interaction between PPTg-nCh and PPTg-Ch neurones is fundamental to response acquisition. Of course, deficits could also be obtained following both QUIN and IBO even if the PPTg-nCh was not directly required for CR lever-press acquisition, but the interaction between PPTg-nCh and PPTg-Ch neurones was essential. However, given that one role which has been put forward for the PPTg-Ch is to incorporate associations made in the PPTg-nCh with forebrain activity, such an interpretation would be absurd. Therefore if the PPTg-nCh is required only when more than one novel stimulus must be tagged simultaneously to one reward, then it would be unlikely that either excitotoxin would be effective in impairing responding for CR.

Therefore in this study, the ability of either QUIN or IBO PPTg-lesioned rats to acquire a lever-press response for CR following AMPH injections into the NAcc was ascertained. To ensure that the initial pairing between the light/click stimulus and reward was established, all rats were given extensive training in this phase of the paradigm prior to surgery. Training sessions were also carried out after recovery from surgery in order to confirm that all rats retained information relating to the CR stimulus.

Methods

Animals

22 male Lister hooded rats with mean body weight $256.7 (\pm 3.50 \text{ [SD]})$ g at the time of surgery.

Surgery

Rats were anaesthetised with Avertin and a unilateral lesion (2 injections) was made in the PPTg with QUIN, IBO or phosphate buffer vehicle. 48 h later rats were re-anaesthetised with Avertin and a second injection made to the opposite side, followed by implantation bilaterally with stainless steel guide cannulae

positioned above the NAcc. All rats were given 14 days to recover from surgery prior to re-commencing training.

Intracranial microinjection

All rats were given bilateral injections of saline vehicle and *d*-amphetamine sulphate (10, 20, 30 µg) during the testing phase of the experiment.

Behavioural testing procedure

Rats were trained in the conditioned reinforcement paradigm and this training recommenced 14 days after surgery. Post-operative training was given for 8 sessions, after which all rats were transferred to the testing phase.

Histological Analysis

Rats were sacrificed between 49 and 53 days after surgery. Parallel sections every 200 µm were processed for NADPH-diaphorase enzyme histochemistry and Nissl substance (cresyl violet).

Statistical Analysis

Ibotenate cell counts and lesion volumes were analysed by t-test to examine whether there were overall differences between left and right sides or between lesions made on the first and second day of surgery. For behavioural data during the training phase, the latency to respond to the CR presentations was recorded and compared by ANOVA before and after surgery. During the test phase total responses on each lever, total panel pushes and frequency of CRs were recorded. Lever-press and panel-press responses were analysed by parametric analysis of variance, with the data subjected to a square-root transformation to achieve homogeneity of variance as recommended by Winer (1971). Untransformed data were also analysed and results from each form of data analysis were similar.

Results

Despite staggering the surgical procedure and leaving 48 hr between operations, 3 rats died following surgery. After the second operation, 1 rat in the IBO group did not recover at all from the anaesthetic and 2 rats (1 IBO and 1 QUIN) died a few days later.

Histological Analysis

NADPH diaphorase-positive cell loss was calculated for each rat and this information is shown in Figure 10:1. Obviously the QUIN lesions in this experiment are very small indeed, but as these rats do all have some cell loss in the region of the PPTg, making them different to control rats they were not discarded from subsequent analyses. Figure 10:2 illustrates microinjection placements from all lesion groups: all rats had injections located in the NAcc.

Figure 10:3 illustrates the largest and smallest lesions in the IBO-lesioned group. Table 10:1 presents the modal damage scores in structures adjacent to the PPTg and the mean lesion volume for an IBO lesion in this study. Damage profiles following IBO injections were similar to those previously obtained using this technique (Dunbar *et al.*, 1992; Chapter 9) and Figure 9:3B is as representative of IBO lesions in this study as it was for the previous one. Analysis of side of lesion showed that there were no differences in diaphorase-positive cell counts or lesion volumes on each side ($t=0.887$ $df=5$ and $t=0.984$ $df=5$ respectively). However, analysis of the order in which the lesions were made demonstrated that the lesions which were made on the first day of surgery killed significantly fewer cells and were significantly smaller in volume than those made subsequently ($t=4.07$ $df=5$ $p<0.01$ and $t=6.71$ $df=5$ $p<0.001$ respectively).

Figure 10:1

Mean (\pm SE) percent NADPH diaphorase-positive cell loss on left and right sides in IBO- and QUIN-lesioned groups.

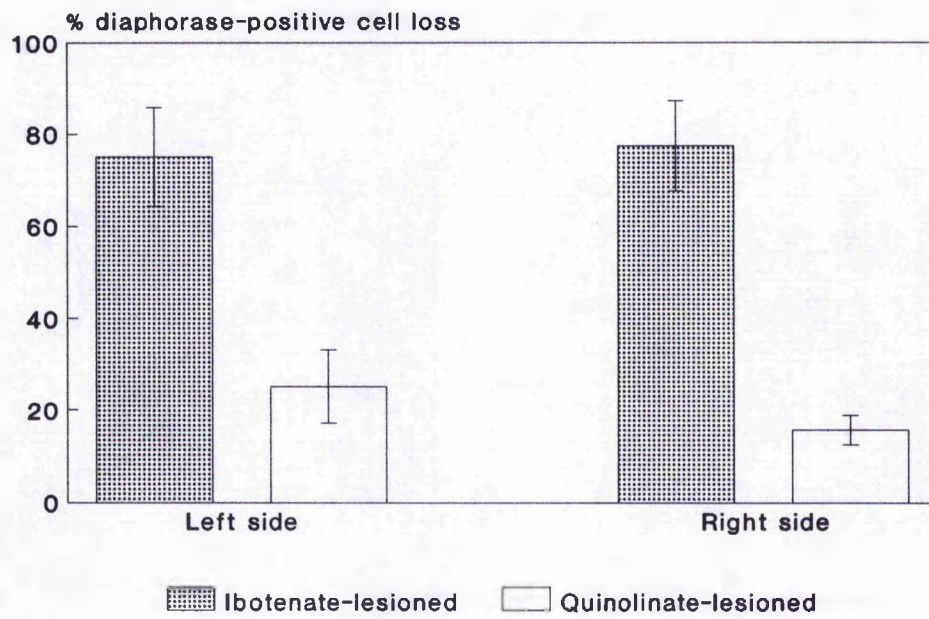
Figure 10:2

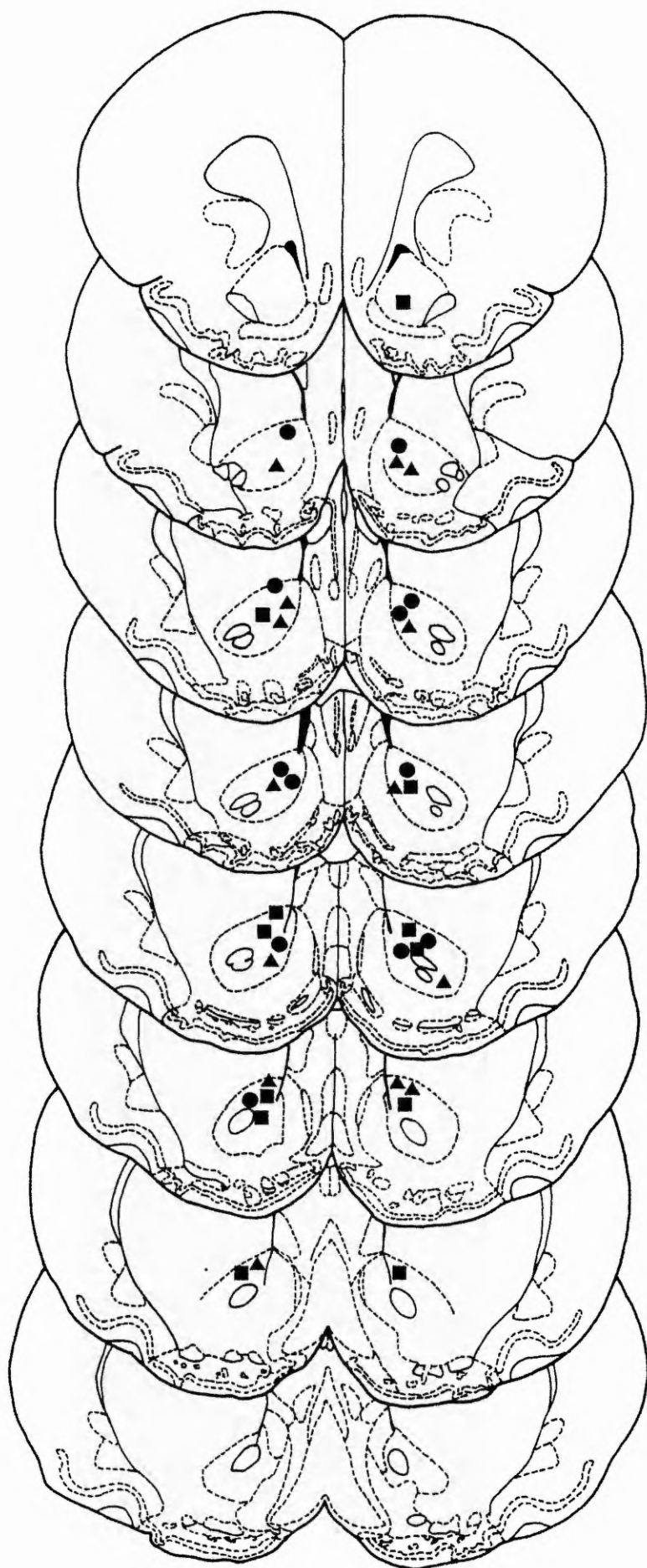
Representative sections, redrawn from the atlas of Paxinos and Watson (1986) showing microinjection placements in the nucleus accumbens. The placements of cannulae in the separate lesion groups are shown by separate symbols (filled circles: ibotenate; filled triangles: quinolinate; filled squares: control).

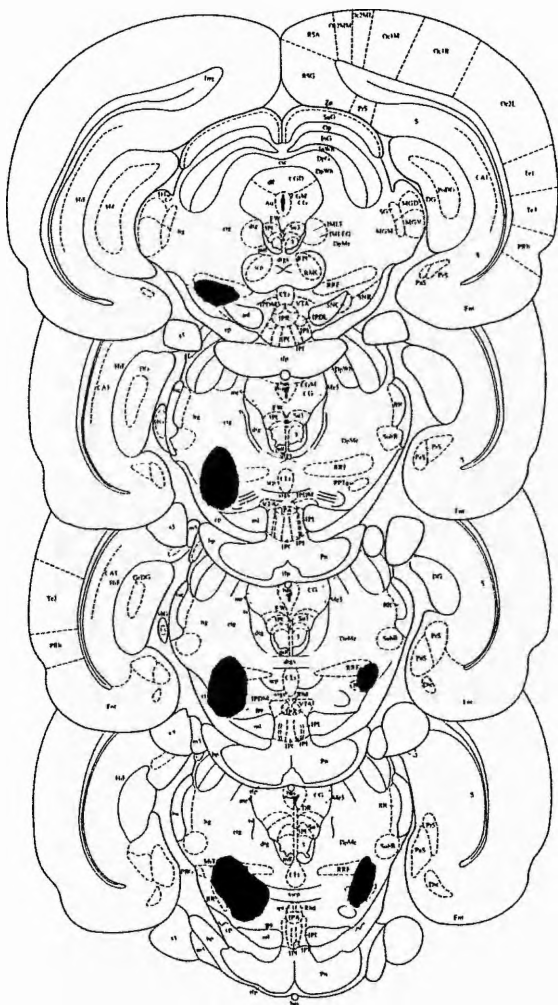
Figure 10:3

Representative sections re-drawn from the atlas of Paxinos and Watson (1986) illustrating the largest (left) and smallest (right) lesion sizes for the ibotenate group.

Figure 10:1







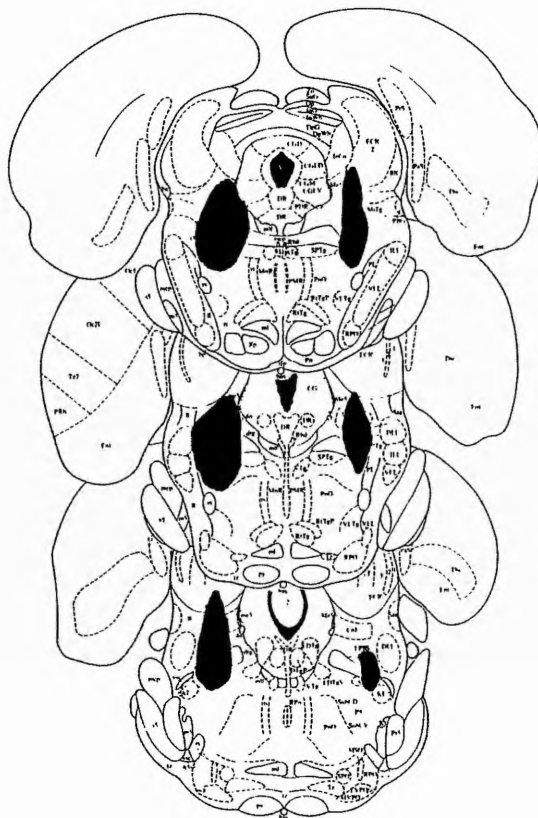
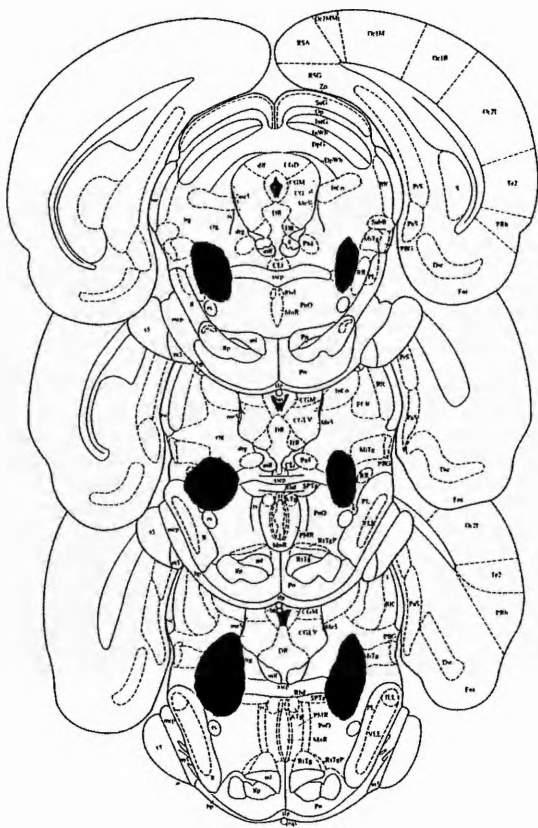


Table 10:1

A summary of damage and average lesion volumes computed from the cresyl violet sections for IBO-lesioned rats. Damage was identified by glial infiltration and degeneration of neuronal somata and a summary of affected structures for every rat was estimated. The modal damage score for the lesion is tabulated for each structure. The key is as follows:

ND	No damage
x	< 30%
xx	30 - 60%
xxx	60 - 90%
xxxx	> 90%

Lesion placement:	Left	PPTg	Right
Damage assessment: (modal scores)			
Parabrachial nucleus	xx		xx
Cuneiform nucleus	xx		xx
Superior cerebellar peduncle	xx		xx
Pedunculopontine tegmental nucleus	xxxx		xxx
Microcellular tegmental nucleus	xx		xx
Subpeduncular tegmental nucleus	x		x
Paratrochlear nucleus	ND		ND
Dorsal tegmental tract	xx		xx
Central tegmental tract	xx		xx
Lateral tegmental tract	x		ND
Deep mesencephalic nucleus	xx		xx
Central gray	x		x
Superior colliculus	track		track
Oral pontine reticular nucleus	x		x
Rubrospinal tract	ND		ND
Retrorubral nucleus	xx		x
Retrorubral field	xxx		xxx
Substantia nigra	x		x
Lesion volume (mm³): (mean + SE values)	3.776 +0.61		3.798 +0.71

Behavioural analysis

Training phase. Latency to respond to the CR light/click stimulus was analysed for 5 days before and 8 days after surgery. There were no pre-operative differences between the groups ($F=0.92$ $df=2,16$), there was no main effect of days ($F=0.72$ $df=4,44$) and no interaction ($F=0.98$ $df=8,44$). However, post-operatively (Figure 10:4) there was a main effect of lesion ($F=11.81$ $df=2,16$ $p<0.001$) although no main effect of days ($F=0.48$ $df=7,112$) or a lesion by days interaction ($F=0.55$ $df=14,112$). *Post hoc* tests revealed that rats in the IBO group significantly increased their latency to respond to the CR stimulus after surgery compared to each other group of rats (both $p<0.005$). It was also noted that the rats in this group began omitting trials after surgery: if the rat did not respond to the light/click within 5 sec by pressing the food-hopper panel to gain access to the food, the light automatically switched off and the computer recorded a missed trial. Table 10:2 clearly shows that while control and QUIN-lesioned rats rarely omitted trials (12 responses out of 56 sessions [~ 70 trials in each] were skipped for the QUIN group; 5 responses out of 48 sessions for controls) the IBO group often neglected to respond to the compound stimulus. Because rats in this group continued to omit trials with similar frequency on consecutive training days (see Table 10:2 - the mean omission rate plateaus) with no apparent sign of improvement, they were transferred to the testing phase with the other groups after 8 days of post-operative training.

Testing phase. It was exceptionally difficult to change stylets or insert microinjection needles into the guide cannulae of rats from the IBO-lesioned group, as they would twist their head back sharply whenever stylets or needles were brought towards or inserted into their head. In addition, all rats from this group (but none from QUIN-lesioned or control groups) were observed grinding their teeth and chewing the edge of the restraining cloth or experimenter's lab-coat while stylets were being changed or injection-fluid infused.

Table 10:2

Mean number of omissions (no response within 5 sec of the compound stimulus presentation) for each group on each post-operative training day. There were ~70 presentations of the compound stimulus in each session.

Training day:	1	2	3	4	5	6	7	8
Ibotenate	7.8 ± 2.9	10.3 ± 4.5	12.8 ± 3.7	12.0 ± 4.0	16.0 ± 5.8	17.0 ± 7.0	15.3 ± 6.5	16.8 ± 7.4
Quinolinate	0.7 ± 0.6	0.7 ± 0.5	0.3 ± 0.3	0.1 ± 0.1	0.3 ± 0.3	1.1 ± 1.1	0.4 ± 0.3	0.7 ± 0.5
Control	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.3	0.4 ± 0.3	0.0 ± 0.0

ANOVA of all data from the test phase revealed no main effect of lesion ($F=0.69$ $df=2,16$) but there was a significant main effect of lever/panel choice ($F=9.43$ $df=2,32$ $p<0.001$) and dose of amphetamine infused ($F=14.11$ $df=3,48$ $p<0.001$). Responses following AMPH were all significantly greater than following saline (all $p<0.05$) (Figures 10:5 and 10:6). There were also interactions between lesion group and lever/panel choice ($F=11.61$ $df=4,32$ $p<0.001$) and between dose and lever/panel choice ($F=2.45$ $df=6,96$ $p<0.05$) but not between lesion group and dose ($F=1.19$ $df=6,48$). The 3-way interaction was not significant ($F=1.66$ $df=12,96$).

Separate analysis of lever- and panel-pressing showed that there were significant effects of dose on each lever (CR lever: $F=10.99$ $df=3,48$ $p<0.001$; NCR lever: $F=6.48$ $df=3,48$ $p<0.005$) and on the panel ($F=8.17$ $df=3,48$ $p<0.001$). Although there were no significant effects of lesion or a lesion by dose interaction related to CR lever-pressing ($F=0.02$ $df=2,16$ and $F=0.76$ $df=6,48$ respectively) or panel-pressing ($F=2.5$ $df=2,16$ and $F=0.41$ $df=6,48$ respectively) there was a lesion effect and a lesion by dose effect related to NCR lever-pressing ($F=11.19$ $df=2,16$ $p<0.005$ and $F=13.78$ $df=6,48$ $p<0.01$ respectively). *Post hoc* analysis demonstrated that the IBO-lesioned group pressed more on the NCR lever at all doses of AMPH than following saline (all $p<0.05$) and that this group also pressed more on this lever following all doses of AMPH than did rats in either the QUIN or control groups following saline or AMPH (all $p<0.001$).

Lever-pressing data from the IBO group were compared further to investigate whether CR and NCR lever-pressing scores in this group were significantly different. There was a main effect of dose ($F=7.84$ $df=3,15$ $p<0.001$), but no main effect of lever ($F=1.07$ $df=1,5$) and no lever by dose interaction ($F=1.03$ $df=3,15$).

Figure 10:4

Mean (\pm SE) post-operative latencies to respond to the CR stimulus on each training day. The ibotenate-lesioned rats were significantly slower to respond to the light/click stimulus than either controls or quinolinate-lesioned rats (see text for details).

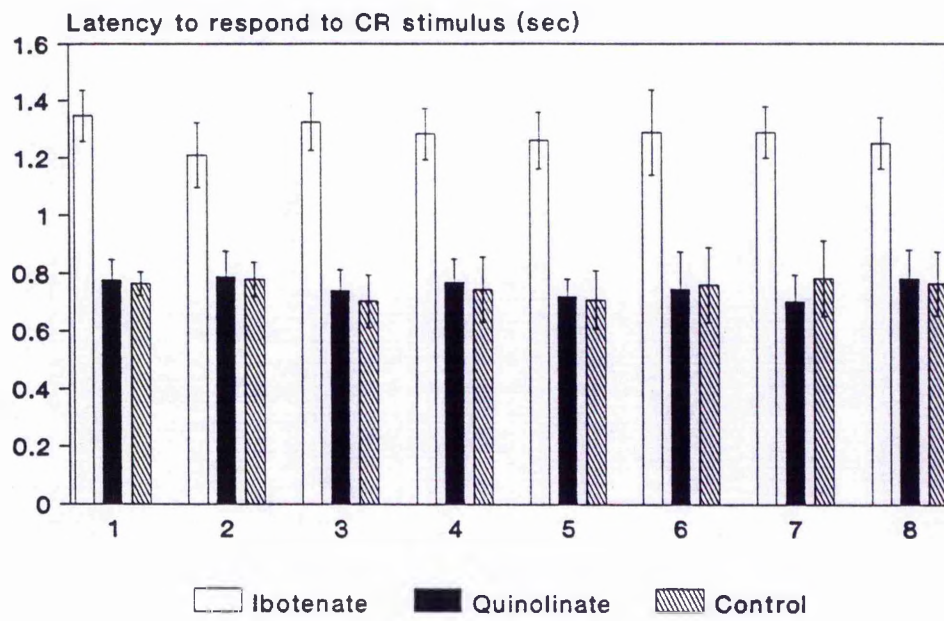
Figure 10:5

A comparison of responding on the CR / NCR levers and the panel giving access to the food hopper in the control and QUIN-lesioned groups following saline and each dose of AMPH during the test-phase. Data were square-root transformed but the actual values are shown.

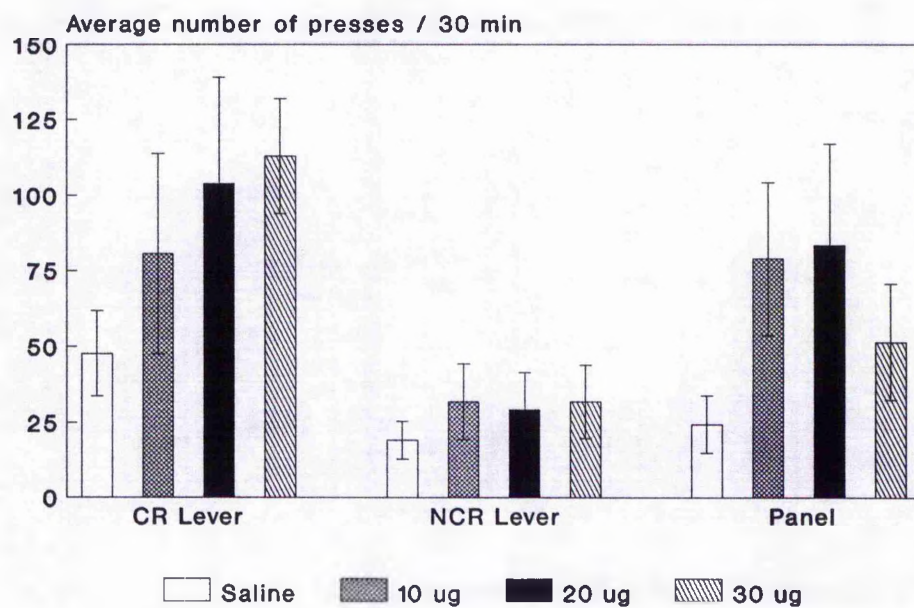
Figure 10:6

A comparison of responding on the CR / NCR levers and the panel giving access to the food hopper in the control and IBO-lesioned groups following saline and each dose of AMPH during the test-phase. Data were square-root transformed but the actual values are shown.

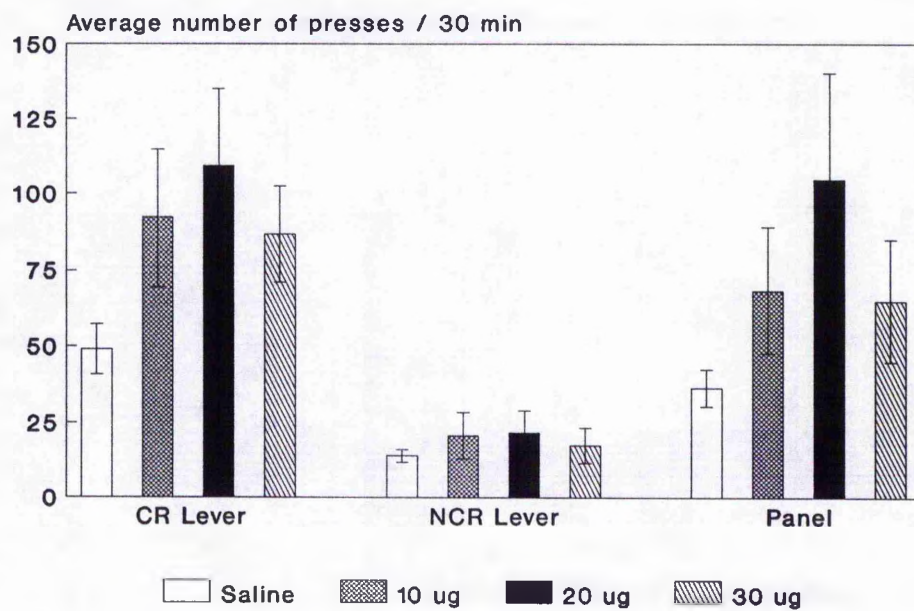
Figure 10:4



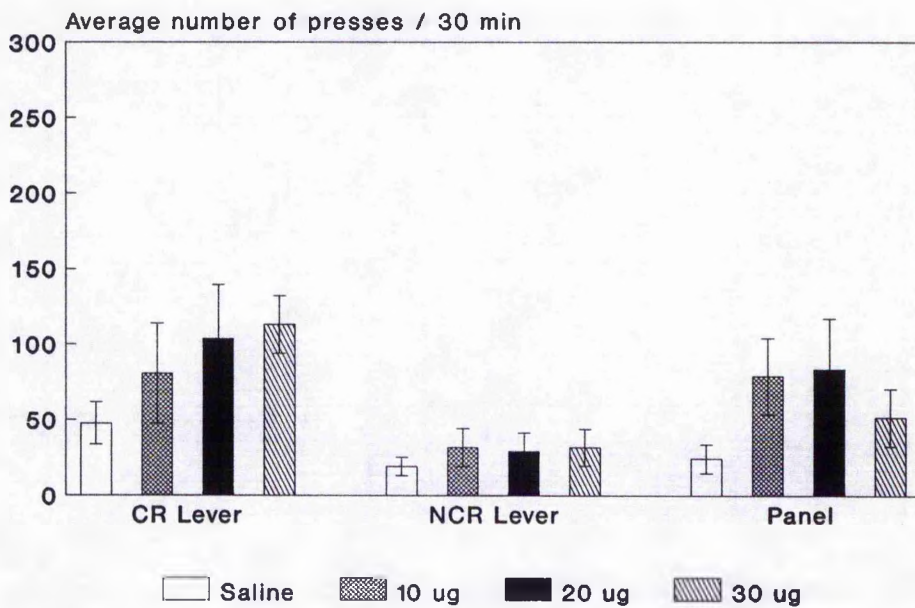
Control



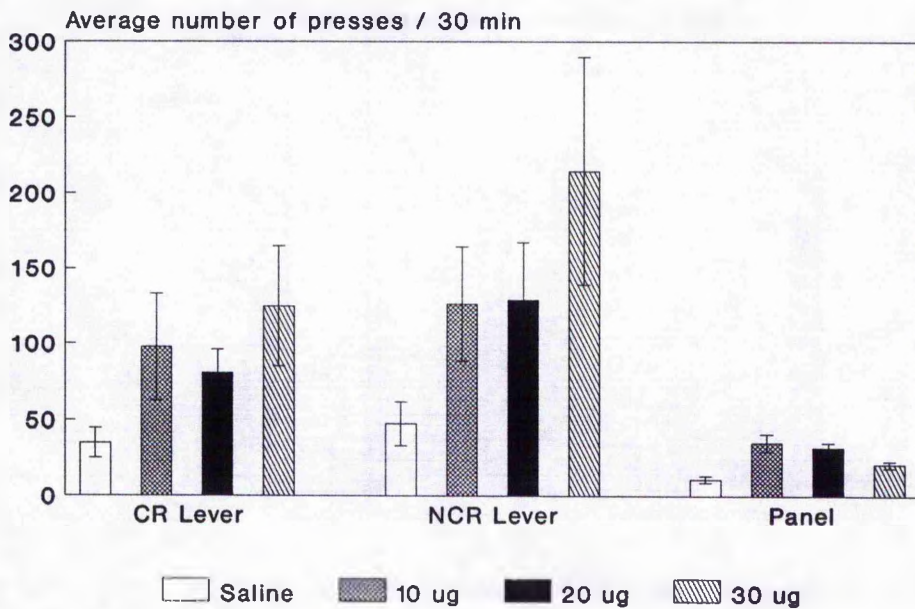
Quinolinate-lesioned



Control



Ibotenate-lesioned



Discussion

These data demonstrate that rats with bilateral PPTg IBO lesions respond differently to controls in the AMPH-stimulated CR paradigm. In the post-operative training phase, IBO-lesioned rats were slower to respond to the presentation of the conditioned stimulus and food. In fact they omitted to respond at all to many of these presentations. In the test phase, this group pressed the CR lever as often as controls, but responded equally on the CR and NCR levers. Panel-pressing in the IBO-lesioned rats was not different to control. QUIN-lesioned rats were not impaired in their responding during either post-operative training or test phases, but histological analysis revealed that damage to the PPTg-Ch in this group was scarce. In fact most of the QUIN-lesioned rats lost no more than 25% of diaphorase-positive neurones in the PPTg.

Quinolate-lesioned rats

The reason for the poor lesion obtained in this case from QUIN infusions is unclear. However, it has been observed previously that QUIN lesions of the PPTg are sensitive to the type of anaesthesia used: sodium pentobarbitone decreased the diaphorase-positive cell loss following 24 nmol QUIN from an average of 75% to 30% (Inglis *et al.*, 1993). Also, although under sodium pentobarbitone anaesthesia 30 nmol QUIN destroyed 60% of these neurones, the relative specificity for PPTg-Ch neurones was lost (Inglis *et al.*, 1993). In the same way, the specificity for these neurones declined as the concentration of QUIN was increased using Avertin anaesthesia (Dunbar *et al.*, 1992). These data emphasise the variable nature of the excitotoxic effects of QUIN in the PPTg, highlighting not only that its specificity is a function of dose, but also that other factors - in this case anaesthetic, but conceivably also the depth of anaesthesia or temperature of the rat - can alter its neurotoxic effects on the diaphorase-positive neurones there.

Although there were no significant deficits in the responses made by the QUIN-lesioned rats compared with those in the control group, the neuronal damage was too small to allow any valid new conclusions to be made regarding the importance of PPTg-nCh/PPTg-Ch interactions in the formation or use of stimulus-reward associations. However, it is worth noting that one rat in this lesion group consistently omitted 10% of the trials in the post-operative training phase, while the others rarely missed any. Although this omission-rate is not as high as was observed from many of the IBO-lesioned rats, this particular rat had one of the largest estimates of diaphorase-positive cell loss in the QUIN-lesioned group, suggesting that PPTg-Ch neurones were responsible at least in part for this deficit.

The role of the PPTg in the formation of stimulus-reward associations

The omission and latency data give the superficial impression that the IBO-lesioned rats found the conditioned stimulus less rewarding than controls. However, previous data suggest that rats with IBO lesions of the PPTg retain incentive associations which have been acquired prior to surgery: Bechara and Van der Kooy (1989) demonstrated that conditioned place preferences learned prior to lesioning were unaffected post-operatively and Fujimoto and colleagues (Fujimoto *et al.*, 1992) found that if intact rats learned the significance of a warning buzzer/light conditioned stimulus in predicting footshock, they could use the predictor to avoid shock after PPTg lesion. In fact, the observation that IBO-lesioned rats did acquire the lever-pressing response in the test phase suggests that they actually did retain the incentive value of the conditioned stimulus. Therefore, rather than displaying a specific deficit in recognising, or acting in response to, a stimulus previously associated with reward, PPTg IBO-lesioned rats may be impaired instead at attending to the stimulus. Retrieving food at the hopper will have become virtually automatic by this stage of training, but efficient responding at the hopper will always require focussing of attentive processes so that valuable reward-presentation time is not wasted by inappropriate responding which delays

delivery of the next trial. Exactly what these "attentive" processes represent is unclear, but they are likely to be related to the ascending reticular function of the PPTg-Ch neurones, which were extensively damaged in these rats.

Although the responding for CR in the IBO-lesioned group was not different to control, this lesion group actually responded equally on CR and NCR levers. Responding on the NCR lever has no programmed effects and is considered to be an indicator of non-specific increases in activity (Robbins, 1978). However, the fact that the IBO-lesioned rats did not respond equally on both the levers and the panel suggests that there was some form of response specificity in their actions. For instance, it is possible that the PPTg-lesioned rats were able to make the lever/reward association but could not dissociate between the levers. This interpretation would suggest that the PPTg-nCh is required only when a concurrent association must be made between more than one novel stimulus and one motivational indicator. In the acquisition of CR, both levers must be linked to the same motivational indicator (the conditioned stimulus reward) although one is a positive association (CR lever-press → reward) and the other is a negative association (NCR lever-press → no reward). The IBO-lesioned rats do not appear to be able to separate these connections. It appears, however, that the PPTg-nCh is not required to make an association between one stimulus and reward, because the IBO-lesioned rats did appear to acquire the general lever-press/reward relationship. This hypothesis suggests that PPTg IBO-lesioned rats would be able to acquire the association between the light/click and reward in the CR training phase and this would merit further investigation.

The observation that IBO-lesioned rats would flinch more than other rats when changing stylets or microinjecting, may relate to the suggested involvement of the PPTg in nociception (Chapter 4). The PPTg clearly receives information relating to painful stimuli (Iwamoto, 1989; Iwamoto, 1991), although it is the moderation of

the response to such stimuli rather than pain itself which is likely to be mediated there (Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992). Indeed the frequent startle response observed during stylet-changing and microinjection in IBO-lesioned rats may be suppressed normally by mechanisms in the PPTg, in a similar manner to the suggested mediation of the acoustic startle reflex by pre-pulse inhibition at this site (Leitner *et al.*, 1981). That these rats also exhibited inappropriate oral-motor behaviour at such times is further evidence for some form of representation of oral information at this site.

Conclusions

The data presented in this Chapter demonstrate that:

1. PPTg ibotenate-lesioned rats omit more trials and are slower to respond to the CR stimulus than controls, suggesting that they are impaired at attending to routine stimuli.
2. PPTg ibotenate-lesioned rats learn the lever/reward association but cannot discriminate between the levers. This suggests that the PPTg-nCh is not required to make an association between one stimulus and reward, but is required instead where concurrent associations must be made between more than one novel stimulus and one motivational indicator.
3. The excitotoxic effects of quinolinate on PPTg-Ch neurones may be exceptionally susceptible to fluctuations in temperature or depth of anaesthesia.

11. General Discussion

Methodological considerations

Microinjections: fluid diffusion from the injection site

The term "microinjection" is routinely used to describe the injection of microlitre volumes into central tissue for anatomical, physiological and behavioural studies. Data obtained following injections into discrete brain regions are presumed to be due to specific actions of the injected drug at that site. However, such interpretations should be justified by information related to concentration gradients and the spread of injected fluids in central tissue at different time-points post-injection.

Estimation of fluid diffusion following intracerebral injections has been investigated in the rat by injecting dyes of different molecular weights in various microlitre volumes (0.5 - 4.0 μ l) into the thalamus or hypothalamus (Myers, 1966). Although the dyes diffused to different extents, the pattern of diffusion for 0.5-2.0 μ l injections was always tear-drop or spherical and there were no measurable differences in the extent of diffusion in either structure. Substances with the largest molecular weights seemed to diffuse least, but other factors such as solubility, pH, osmolarity and electrovalent characteristics also contributed to the magnitude of spread of each solution.

More recently, the 3-dimensional distribution gradient of tritiated DA injected into the dorsal striatum was estimated in the primate (Dubach, 1991). The initial spread of fluid was consistent with distribution by pressure: the injection fluid (1 μ l) rapidly filled the extracellular space within about 1.5 mm of the site and spread furthest in the dorso-ventral dimension. Although the first 10-20 min post-injection were marked by a rapid 25% loss of DA, probably by drainage into broken capillaries, during the next 40-50 min the dimensions of the injection site gradually

expanded and there was minimal loss of label. Fluid dispersion appeared to be guided by the direction of axon bundles and blood vessels as it spread very little in the anterior-posterior dimension and is therefore guided by the tortuosity of the tissue and existing bulk flow of interstitial fluids.

These studies imply that the volume and rate at which fluid is infused should be appropriate to the size and organisation of structure into which the injection is made. Injections of 0.5 μ l infused at a rate of 1.0 μ l / 43 sec will produce an average diffusion volume of 1 mm, while 2.0 μ l infused at the same rate will fill a volume of approximately 2.5 mm (Myers, 1966). Although the rates of infusion in the current studies (0.5 μ l/min) were different to those used by Myers (1966), the analogous volumes give these figures direct relevance. Using these diffusion volumes as approximate guides, it is evident that reasonable placements at each site will produce excitation relatively well-restricted to SNc (0.5 μ l), VTA (0.5 μ l) and NAcc (2.0 μ l).

Excitotoxic lesions: Demyelination of fibres of passage

Although the use of excitotoxins to make fibre-sparing lesions in the central nervous system is now well-established (Winn, 1991), recent descriptions of the cascade of pathological events which occur following excitotoxic infusions (Coffey *et al.*, 1988; Coffey *et al.*, 1990) have cast doubt over the fibre-sparing nature of the tissue damage. It has been determined that injections of IBO into the septum not only destroy neurones in the immediate vicinity of the infusion, but also lead to a proliferation of "non-neuronal" matter there (Coffey *et al.*, 1988). Although this microglial invasion is most likely part of the inflammatory response associated with the removal of neuronal waste from the damaged region, mechanisms associated with their presence at the lesion site may also induce non-specific damage such as the demyelination of fibres of passage (Coffey *et al.*, 1988).

The presence of reactive glial cells such as macrophages at the site of the lesion was linked directly to the extended breakdown of the blood-brain barrier (BBB) which occurred following a 0.2 μ l injection of 2 μ g IBO, but not saline vehicle, into the septum (Coffey *et al.*, 1990). The BBB was still permeable to horseradish peroxidase (HRP) 7 days after the lesion and the spread of HRP reaction product matched the region of intense reactive gliosis (Coffey *et al.*, 1990). However after carrying out an irradiation procedure to deplete the progenitor cells in the bone-marrow, BBB breakdown at the lesion site was diminished and demyelination of fibres of passage was repressed (Coffey *et al.*, 1990).

It is almost certain that the occurrence of demyelination is a general phenomenon following injection of any excitotoxin into any part of the CNS. The fact that septal fibres are present in small, dispersed bundles may make them particularly vulnerable. Indeed in the striatum where fibres exist instead in large bunches, melomonocytic cells do not invade them and the myelin sheaths remain more or less intact (Coffey *et al.*, 1988). Therefore it appears that myelin may only be damaged where degenerating neurones and fibres are interspersed. Indeed it is known that kainate infusions into the thalamus (Dusart *et al.*, 1992) and both IBO (Stellar *et al.*, 1991) and NMDA (Brace *et al.*, 1992) infusions into the lateral hypothalamus demyelinate fibres of passage.

Evidence for remyelination. My colleagues have recently observed remyelination of fibres in rat lateral hypothalamus, beginning between 14 and 21 days after infusions of 90 nmol NMDA (Brace *et al.*, 1992). Furthermore other researchers have demonstrated that remyelination can begin to occur 1 month after lesions were made in the rat thalamus with 5 nmol kainate (Dusart *et al.*, 1992). In the lateral hypothalamus (Brace *et al.*, 1992), small grains of myelin were often identified 14 days post-lesion and by 21 days these fragments were widespread and typically associated with blood vessels. New strands of myelin did not creep in

from the edges but instead could be seen passing through the entire lesioned region. Remyelination was essentially complete by 3 months.

Implications for PPTg and DpMe lesions. Preliminary analyses of IBO-induced damage in the PPTg suggest that demyelination of dispersed fibres does occur there, but that the myelin sheaths in compact bundles remain intact (H. Brace, M. Latimer and P. Winn, unpublished observations). This is further evidence for the particular vulnerability of individual fibres and complements data previously obtained for large bundles of striatal neurones versus more scattered septal fibres (Coffey *et al.*, 1988). Previous histological observations in this laboratory also indicated that TOH-positive fibres remain intact following IBO lesions of the PPTg (Rugg *et al.*, 1992), demonstrating that fibre damage *per se* does not occur. Furthermore, preliminary data suggest that where myelin is lost in the PPTg, remyelination takes place in a fashion similar to that observed in the lateral hypothalamus (H. Brace, M. Latimer and P. Winn, unpublished observations).

It is unlikely that the behavioural effects observed in this thesis following excitotoxic lesions of the PPTg or DpMe can be attributed to fibre damage. In Chapter 9, although the measurements of food and water intake and locomotion took place in the early post-operative period - the most crucial in terms of the effects of demyelination - they revealed no differences between the lesioned groups and controls. Later in the study, when the effects of AMPH and APO on locomotion and stereotypy were investigated, remyelination would probably have been substantial. In Chapter 10, conditioned reinforcement training was not recommenced until day 15 post-operatively when at least minimal remyelination would have been underway. The latency and omission data in the training phase could be attributed to a slowing of neuronal impulses, which might be expected following damage to myelin sheaths. However, the fact that IBO-lesioned rats made more responses on the levers than controls in the test phase suggests that

these behavioural modifications were due specifically to neuronal damage within the PPTg rather than slowing of responding as a result of myelin-related fibre damage.

Histological analysis

Cell counting technique. Estimation of numbers of ChAT- or diaphorase-positive neurones in the PPTg in control and lesioned brains was carried out in Chapters 9 and 10 by counting cell profiles in 50 μm sections. Because the average diameter of cholinergic PPTg neurones is 20 μm (Rye *et al.*, 1987) and because cell profiles were not counted on adjacent sections, the same neurone cannot be scored more than once and so the profiles counted should not over-estimate the actual neuronal numbers (see Coggeshall, 1992). This method of comparing profile numbers in experimental and control conditions is therefore relatively satisfactory and should not require adjustment by correction factors of the sort outlined by Coggeshall (1992).

NADPH-diaphorase versus ChAT in the PPTg. ChAT-immunohistochemistry identifies the catalytic enzyme ChAT, an essential precursor for the formation of ACh. Labelling of ChAT-positive neurones by the use of a specific antibody which is tagged with a series of indicators and subsequently visualised via a peroxidase reaction product is considered to be the most reliable indicator for the presence of cholinergic neurones throughout the CNS. NADPH-diaphorase is a nitric oxide (NO) synthase responsible for the calcium/calmodulin-dependent formation of citrulline and NO from arginine. It is found in many neurones in brain but is not associated consistently with any specific neurotransmitter. In the pons, cholinergic neurones appear to be almost alone in producing NO. NADPH-diaphorase has been used to label pontine cholinergic neurones (Vincent *et al.*, 1983) in both the rat (Vincent and Kimura, 1992) and human (Mesulam *et al.*, 1989) brain. In a double-labelling study of human brain, Mesulam and colleagues (1989)

demonstrated that all ChAT-positive neurones in the Ch5 and Ch6 region stained for diaphorase, although approximately 10% of diaphorase-positive neurones in the general region of Ch5/Ch6 were ChAT-negative.

In Chapter 9, diaphorase-positive neuronal counts were compared directly with ChAT-positive counts in parallel sections. A statistically significant correlation was found between them, confirming that in the rat brain pontine cholinergic neurones can be acceptably labelled with NADPH-diaphorase. Indeed a number of reasons can be advanced for preferring to use diaphorase histochemistry rather than ChAT in the pons: first, the staining process for NADPH-diaphorase is considerably more straightforward and less time-consuming than the protocol for ChAT; second, less handling of the tissue is required for diaphorase staining, so the sections are less fragile and do not tend to fall apart before being mounted on to the slides; third, visualisation of pontine neurones with diaphorase is always more distinct than with ChAT due to lower background staining, making cell-counts more accurate; and fourth, NADPH-diaphorase histochemistry is cheaper. For these reasons, NADPH-diaphorase is generally the histochemical technique of choice for identification of pontine cholinergic neurones.

Discussion of the control of striatal dopamine by cholinergic neurones in the pons

The data presented in this thesis have important implications for understanding the way in which mesopontine cholinergic neurones influence DA systems. It is well-established that muscarinic activation of SN can dose-dependently increase activities for which the animal has both a low baseline rate and a positive predisposition (Winn and Redgrave, 1979; Winn and Redgrave, 1981; Winn *et al.*, 1983; Winn, 1991). The data presented in Chapter 6 demonstrate that this phenomenon can be extended to include nicotinic, or simultaneous muscarinic and nicotinic, activation by blocking the breakdown of endogenous ACh using

neostigmine. In Chapter 7, increased feeding following neostigmine, but not baseline feeding, was blocked by either nicotinic or muscarinic cholinergic antagonism, suggesting that innervation of the SNc is phasic rather than tonic in nature. The data presented in Chapter 8 demonstrate that the behaviours which can be induced by cholinergic stimulation of SN do not only include consummatory responses, but also encompass acquisition of responding for CR. The low current baseline rate and positive predisposition also appear to be relevant to the acquisition of responding for CR. Well-trained rats learn that lever-pressing is superfluous to gaining reward and therefore have a low baseline rate of lever-pressing when they enter the testing phase. In addition, the rats in this study have a positive predisposition to acquire the response: if they had been given a random pairing between the light/click stimulus and food presentation in the training phase, they would not have acquired the lever-press response in the test phase (see Cador *et al.*, 1991). Therefore, it is a low rate, positively predisposed behaviour that is enhanced by cholinergic stimulation of SN.

The absence of CR acquisition following VTA stimulation by neostigmine is unlikely to mean that mesolimbic DA is not required for CR acquisition. As outlined in Chapter 8, testing at different stages of CR training has produced apparently different results following cholinergic SN or VTA injections. The hypothesis put forward to explain this relates to changes in the spontaneous firing of nigrostriatal *and* mesolimbic DA neurones as performance of a task becomes habit through extensive training (Ljungberg *et al.*, 1992). The proposed effects of cholinergic manipulation at different stages of training are summarised in Figure 11:1. The crucial indicator of whether a rat will acquire the CR lever-press response appears to be the rate of firing of nigrostriatal DA neurones: as long as the rat has at least some appreciation of the incentive value of the environment it will learn an incentive-driven response, provided that the activity of nigrostriatal neurones is sufficient to shape the response. Therefore, stimulation of behaviours

Figure 11:1

A summary of the hypothesis outlined in Chapter 8 describing the differences in the effects of cholinergic manipulation on DA neuronal activity at different stages of learning a novel task. Although the fall off in neuronal firing would be a gradual decline (Ljungberg *et al.*, 1992) discrete points (high, medium and low) have been selected for illustration. Similarly, although neostigmine is specifically mentioned, the hypothesis ought to extend to other cholinergic drugs, such as carbachol and nicotine.

Figure 11:1

	Site	<i>No training</i>	<i>Some training</i>	<i>Exhaustive training</i>
DA firing	SN/VTA	HIGH	MEDIUM	LOW
DA function	SN	Construct response set	Modify existing response set	None: established response set
	VTA	Learn motivational significance	Modify motivational significance	None: established motivational significance
Neuronal effect of neostigmine injection	SN	None	Increase firing of nigrostriatal DA neurones (mesolimbic firing unchanged)	Increase firing of nigrostriatal DA neurones (mesolimbic firing unchanged)
	VTA	None	Increase firing of mesolimbic DA neurones (nigrostriatal firing unchanged)	Increase firing of mesolimbic DA neurones (nigrostriatal firing unchanged)
Behavioural effect of neostigmine injection	SN	None	Construct new response set (incentive still being modified)	Construct new response set (incentive unchanged)
	VTA	None	Learn new motivational significance (response set still being modified)	Learn new motivational significance (response set unchanged)

by cholinergic activation of the VTA will require a separate set of parameters to those established for activation of behaviours by SNc stimulation. Indeed it would appear that a higher baseline rate of activity on the levers - incomplete formation of the response set - may be necessary.

Clearly, cholinergic control of nigrostriatal and mesolimbic DA neurones could be explored further by the use of operant tasks. For instance, if rats were systematically trained for various time periods before transferring to the test phase in the CR paradigm, would the effects of neostigmine stimulation of the VTA or SNc vary with regard to the degree of training? In addition, if hungry rats were trained initially to press a lever for food when a light came on above the lever, and subsequently trained to press the lever *opposite* the light signal, what effect would neostigmine injections into SN or VTA have on response modification? If DA in the CPu is required to define a response set, then one would hypothesise that SN injections would help extensively trained rats to switch their responses (once DA neurones in the CPu are beginning to stop firing) but would not affect rats trained for shorter periods of time to the same extent (as CPu DA would still be active). To put it another way, rats would have a positive predisposition to lever-press for food at any stage of training, but only well-trained rats would have a low current baseline rate of pressing the "wrong lever". Furthermore, the hypothesis outlined in Figure 11:1 implies that in this sort of task neostigmine injections into the VTA might only be helpful for less extensively trained rats.

Discussion of the role of the PPTg in outflow from the striatum

The experiments reported in this thesis have implications for existing theories of the PPTg-nCh as a striatal output station. First, although the image of the PPTg as a locomotor centre still dominates the literature (Garcia-Rill, 1991) much evidence has accumulated in recent years to contest this perspective (Swerdlow and Koob, 1987; Dellu *et al.*, 1991; Olmstead and Franklin, 1992; JS Dunbar and P Winn,

unpublished observations). The data presented in Chapter 9 strengthen the view that the PPTg is not *the* or even *a* site of the mesencephalic locomotor region and should direct future research relating to locomotion towards the adjacent cuneiform nucleus (Eidelberg *et al.*, 1981; Coles *et al.*, 1989; Shojania *et al.*, 1992) and other potential MLR components. Second, evidence pointing to the importance of the PPTg in the integration of incentive information (Bechara and Van der Kooy, 1989; Bechara and Van der Kooy, 1992a; Bechara and Van der Kooy, 1992b; Bechara and Van der Kooy, 1992c; Fujimoto *et al.*, 1989; Fujimoto *et al.*, 1992) has been confirmed and extended in a conditioned reinforcement paradigm (Chapter 10), a test unexplored in this context. Although the previous evidence which pointed to the PPTg as having reward-related functions was very convincing, many of these data did little to define an image for the PPTg except to regard it as a pontine extension of the NAcc. Clearly the PPTg is different to the NAcc both with respect to its anatomical position and the array of information which it receives and distributes. Therefore while the PPTg does appear to have a role in the processing of motivationally significant novel stimuli, the dorsal striatal and basal ganglia outputs it receives in addition to its critical cholinergic component suggest that its purpose must be radically different to that assigned to the NAcc.

Inappropriate oral movements were observed from IBO-lesioned rats in both of these experiments, suggesting that the PPTg normally has inhibitory control over the generation of orofacial activity. Although a functional connection between the dorsal striatum and the PPTg has previously been demonstrated (Mitchell *et al.*, 1989), there was previously no direct experimental evidence for this as an important behavioural output pathway. Indeed it is unlikely that even the present data can be taken to imply that the PPTg is involved specifically in the execution of oral behaviour: specific "oral" nuclei located in the medulla receive innervation directly from the ventrolateral CPu / SNr output pathway (Von Krosigk and Smith,

1990; Von Krosigk *et al.*, 1992) and neurones from these medullary structures project directly to orofacial motoneurons in the spinal cord (Travers and Norgren, 1983). Therefore the PPTg probably has a modulatory role on the expression of orofacial behaviour by medullary neurones. The exact nature of this modulation is unclear: data which implicate the PPTg in the integration of novel stimuli with their motivational significance (Chapter 4; Chapter 10) imply that the PPTg must receive information relating to the "appropriateness" of various actions in given situations. As oral nuclei in the pons may be excited by numerous disparate stimuli, neuronal activity there may require the influence of the PPTg in order to gate firing patterns so that neuronal activity becomes more relevant to the oral requirements of the environment.

The model put forward (Chapter 4) implicating the PPTg in the integration of incentive-related responses (for instance approach of reward, avoidance of shock, modification of reactions to pain [antinociception] or to acoustic startle [pre-pulse inhibition]) is directly relevant to the data relating to involvement of the PPTg in CPu outflow. If the PPTg receives information relating to orofacial activity from the lateral CPu then it must also be in receipt of information relating to forelimb reaching (Pisa, 1988). PPTg QUIN-lesioned rats were unable to reach as far as controls or IBO-lesioned rats in a reaching/grasping task (Dunbar *et al.*, 1992) suggesting that information relating to forelimb muscle control is represented in some form in the PPTg. Indeed muscle tone can be modified directly by stimulation of PPTg neurones (Kelland and Asdourian, 1989). Pre-pulse inhibition is a specific example of the ability of tegmental structures (Leitner *et al.*, 1981) to repress muscle contraction in inappropriate circumstances. In the same way that muscular activity may be modified by PPTg neurones in pre-pulse inhibition, oral activity may be directly suppressed by neurones in the PPTg in situations unsuitable for such behaviour.

The hypothesis, that a role for the PPTg-nCh is in the concurrent association of more than one novel stimulus with one motivational indicator to produce an appropriate response, should be investigated further. For instance, would a rat with an IBO lesion of the PPTg be able to acquire the association between the light/click and reward in the CR training phase? If so, would a hungry PPTg IBO-lesioned rat learn a lever-press response for food? The hypothesis suggests that they would accomplish the former response, but would not be able to dissociate between the levers in the latter task. However, it would be particularly interesting to discover whether PPTg IBO-lesioned rats would be able to dissociate between the levers if one delivered food and the other delivered a footshock: acquisition of the rewarding and aversive lever-press associations might have to be staggered, but since they may be represented on separate motivational indicators dissociation between the levers may be possible.

The role of the PPTg-Ch may also be understood better through such experiments. The pattern of deficits which would be obtained following QUIN lesions in both the CR paradigm and the experiments outlined above is unclear for two reasons: first, the extent to which PPTg-Ch neurones are required for processing of motivationally relevant associations is unknown; and second, QUIN does damage PPTg-nCh neurones even if the extent of the damage to this part of the PPTg is less than that seen following IBO (Rugg *et al.*, 1992). A different lesion approach may be required in order to answer this. For instance, if it is possible to target the specific antigens present in neurones with antibodies tagged with ricin (Burlet *et al.*, 1992), then PPTg-Ch neurones might be killed most selectively by injecting a specific anti-ChAT/ricin "bullet" into the region of the PPTg.

As described in Chapter 1, some researchers have chosen to define the PPTg as a nucleus comprised entirely of cholinergic neurones, while the non-cholinergic neurones which are interdigitated with them are described separately as the MEA

(Rye *et al.*, 1987; Hallanger and Wainer, 1988; Lee *et al.*, 1988; Steininger *et al.*, 1992). Although this is a valid dissociation to make in terms of the neurochemistry and connectivity of the neurones in this region, it unfortunately obscures the functional importance of their interdigitation. In Chapter 2 an analogy was drawn between the organisation of the SN and the PPTg: Just as interactions between the SNc and SNr are likely to be important, the integration of neuronal information between the PPTg-Ch and PPTg-nCh and modulation of the other's firing patterns is crucial for appropriate behavioural outputs. However, although the SN and PPTg can be viewed similarly for illustrative purposes, their connections dictate that their functions will be very different. While the PPTg-Ch widely innervates both higher and lower structures, the SNc only funnels information forward to the striatum. Similarly, while the PPTg-nCh just directs descending innervation to medullary and spinal centres, the SNr returns partially processed signals to their cortical origins via the thalamus in addition to distributing a basal ganglia plan for action to the superior colliculus and mesopontine tegmentum. Not only are interactions between PPTg neurones likely to be central to a fuller understanding of its functions, but they may also provide important clues of a more general nature for the rules by which ascending and descending signals modify ongoing firing patterns and influence behaviour.

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